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APLASTIC ANEMIA

WITH SPECIAL REFERENCE TO THE SIGNIFICANCE OF THE SMALL LYMPHOCYTES

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In two earlier papers [1] an effort was made to apply the concept of the lymphocyte as an ancestor of the red cell, derived from a comparative study of the development of blood in vertebrates, particularly in amphibians [2] and birds,[3] to an interpretation of histologic observations in certain leukemias. Conditions in the lymph nodes, spleen and bone marrow were shown to be consistent with the idea that in man, as in amphibians and birds, the small lymphocytes after a period of growth serve as hemocytoblasts from which differentiate both erythrocytes and granulocytes. In the present paper histologic observations in a clinically typical case of aplastic anemia will be examined with the aid of this same concept.

Interest centers, therefore, on the role of the small lymphocyte. Accordingly, this study is concerned chiefly with the comparative histologic and cytologic studies of the spleen, lymph nodes and bone marrow. The tissues in this case present a favorable opportunity for further investigation of the possible genetic significance of the small lymphocytes in relation to the production of erythrocytes. The problem under consideration concerns primarily not the cause nor even the histologic appearances of aplastic anemia but the function of the lymphocyte in relation to hemopoiesis.

Only the directly pertinent data in the clinical history and autopsy report need be cited here. The patient, a white woman 56 years of age, was first admitted to the University of Virginia Hospital Aug. 28, 1937. The hemoglobin content of the blood was 10 per cent (Dare). The red cell count was 810,000; the leukocyte count was 2,700 (polymorphonuclears, 49 per cent; small lymphocytes, 47 per cent); the reticulocyte count was 1 per cent; the platelet count, 20,200; there were a few normoblasts. After five blood transfusions had been given in addition to iron and liver therapy, the hemoglobin content rose to 48 per cent, the red cell

From the University of Virginia.

1. Jordan, H. E.: Arch. Path. **18**:1, 1934; **23**:653, 1937.
2. Jordan, H. E., and Speidel, C. C.: Am. J. Anat. **46**:55, 1930.
3. Jordan, H. E.: Am. J. Anat. **59**:249, 1936.

count to 2,110,000 and the leukocyte count to 3,000 (small lymphocytes predominating—66 per cent). The patient was discharged from the hospital September 23. She was readmitted October 18. The hemoglobin content was 30 per cent; the red cell count, 1,250,000; the platelet count, 91,000. The clotting time was eight minutes. There was bleeding from the gums and buccal membranes, and purpuric areas had appeared over the skin. After three blood transfusions the hemoglobin content November 1 had risen to 65 per cent, the red blood cell count to 3,000,000 and the leukocyte count to 4,000 (small lymphocytes predominant—65 per cent). Two blood transfusions were given November 8 and 14, respectively. November 24 the red cell count had dropped to 790,000, the hemoglobin content to 22 per cent and the platelet count to 10,000. The patient became irrational and died the following day, November 25.

The lymph nodes removed at autopsy included cervical, peritoneal and periaortic nodes. The lymph nodes were not enlarged. The spleen weighed 120 Gm. and measured 12 by 6.5 by 3 cm. It was dark purple, and the capsule was slightly wrinkled. The spleen was rather soft, and the cut surface was dark red, against which large white malpighian corpuscles were conspicuous. Samples of marrow were taken from a vertebra, a rib and a femur. The marrow from the femur was yellow, with many red areas.

LYMPH NODES

The lymph nodes were uniformly small. The cortex had the character of compact lymphoid tissue, with indications of nodules (fig. 1). The nodular areas lacked germinal centers (fig. 2), and some nodules were in early stages of fibrosis. Small lymphocytes predominated, but medium-sized lymphocytes were numerous, and large lymphocytes occurred (fig. 3 *C*). Occasional medium-sized and large lymphocytes were in mitosis. The medullary cords were narrow; the sinuses, relatively wide. Occasional macrophages occurred, with chiefly erythrocyte fragments. Certain areas contained scattered plasma cells, but no Russell bodies were seen. Eosinophils were practically absent, but basophils were relatively numerous, occurring in both cortex and medulla. Sections of a ganglion, mistaken at autopsy for a lymph node, contained large numbers of basophils. The irregular and elongated shape of some of these basophils indicated active ameboid motion. The general appearance of the lymph nodes indicated function approaching exhaustion in an effort to supply small lymphocytes. The presence of the plasma cells suggested an abortive effort at local production of erythrocytes; that of the basophilic granulocytes, an abortive effort at production of eosinophils.

The cytologic observations on the several varieties of lymphocytes, granulocytes and plasma cells were identical in lymph nodes, spleen and marrow, and a description of those in the marrow will be included.

SPLEEN

The spleen was of approximately normal size. It was characterized by a great increase in the number of nodules. No nodules with germinal centers occurred. All the nodules were atrophic; a certain number were

sclerotic. Figures 4 and 5 show the average condition; certain nodules were even more diffuse, some slightly more compact. Some of the diffuse variety contained large pale staining mononucleated cells of irregular form. These were potential macrophages but generally lacked

Fig. 1.—Peripheral portion of a vertical section of a cervical lymph node. Just below the capsule are the remnants of four cortical nodules. The hilus is located immediately below the lower border of the figure. The medulla is relatively wide, with cords of fairly compact lymphoid tissue, narrow sinuses and coarse trabeculae. Helly fixation; eosin-azure stain; magnification, 75 diameters.

débris. The structure of these nodules comprised essentially a wide-meshed reticulum with a scattered parenchyma including small, medium-sized and occasional large lymphocytes (fig. 3 *b*). Many of the small

lymphocytes were in process of degeneration. The extranodular parenchyma contained the same variety of lymphocytes, small cells predominating. An occasional medium-sized lymphocyte might be in mitosis.

In certain areas the parenchyma included a considerable number of plasma cells, a few erythroblasts and a few normoblasts. Basophils also

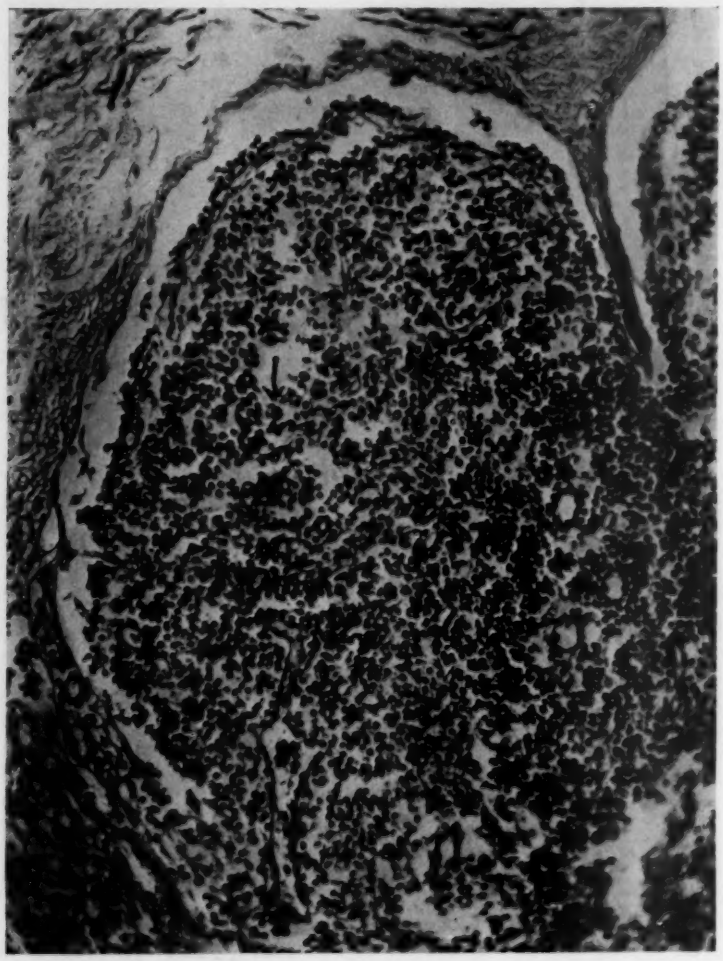

Fig. 2.—Cortical nodule (*X* of fig. 1). There is an indication of a previous "germinal center" in the lighter, looser area near the upper left hand border. Here can be seen a few large lymphocytes (one indicated by the arrow). The bulk of the nodule consists of a fairly compact parenchyma in which small lymphocytes predominate. Helly fixation; eosin-azure stain; magnification, 300 diameters.

occurred but in smaller numbers than in the lymph nodes. No Russell bodies or megakaryocytes were seen. The sinuses were dilated and well

filled with blood, including a few normoblasts. The general appearance of the spleen was that of exhaustion after excessive lymphopoietic activity.

BONE MARROW

The specimens of marrow from the femur, rib and vertebra were essentially identical, i. e., of lymphoid character, and varied in density in different areas of the same section. The chief variations of this

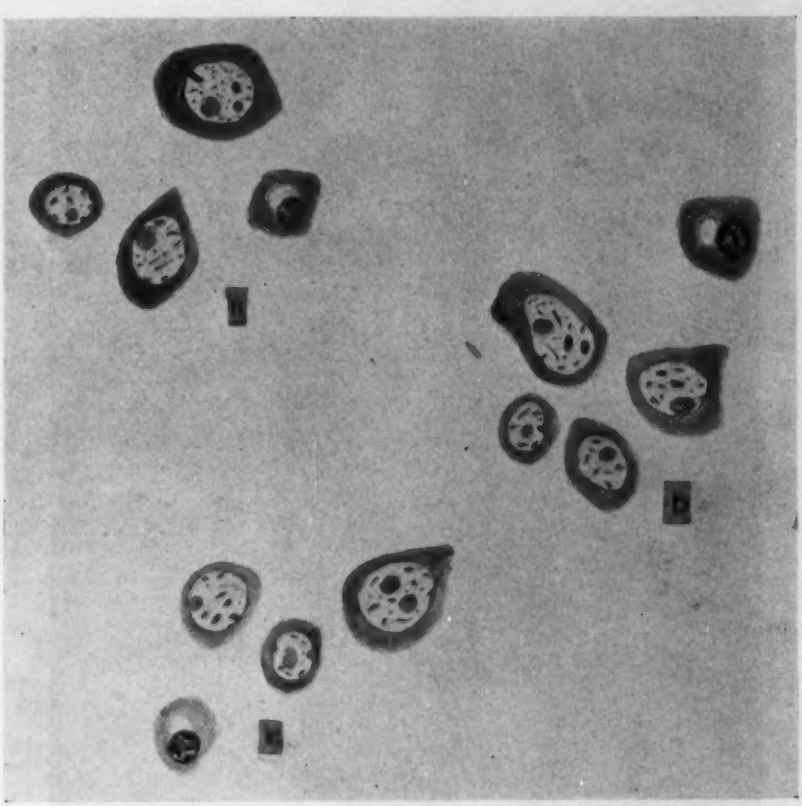

Fig. 3.—Selected cells from average areas of marrow (*a*), spleen (*b*) and cervical lymph node (*c*). Each group includes an essentially identical large lymphocyte (hemocytoblast), a medium-sized lymphocyte, a small lymphocyte and a plasma cell. In preparations treated with eosin-azure stain the cytoplasm of the lymphocyte stains blue, the nucleus light gray and the nucleoli red. Magnification, 1,200 diameters.

lymphoid marrow included (1) a uniformly compact structure, (2) a diffuse structure with considerable fat (figs. 6 and 7) and (3) a diffuse structure with fat and considerable numbers of normoblasts and red blood corpuscles. The small variety of lymphocyte predominated, but

the large variety occurred in much larger numbers than in the lymph nodes and spleen. Occasional large lymphocytes were in mitosis. Certain areas showed masses of lymphocytes, with the larger variety predominating. Examination of these areas with the oil immersion lens revealed that these clumps of lymphocytes were confined in venous

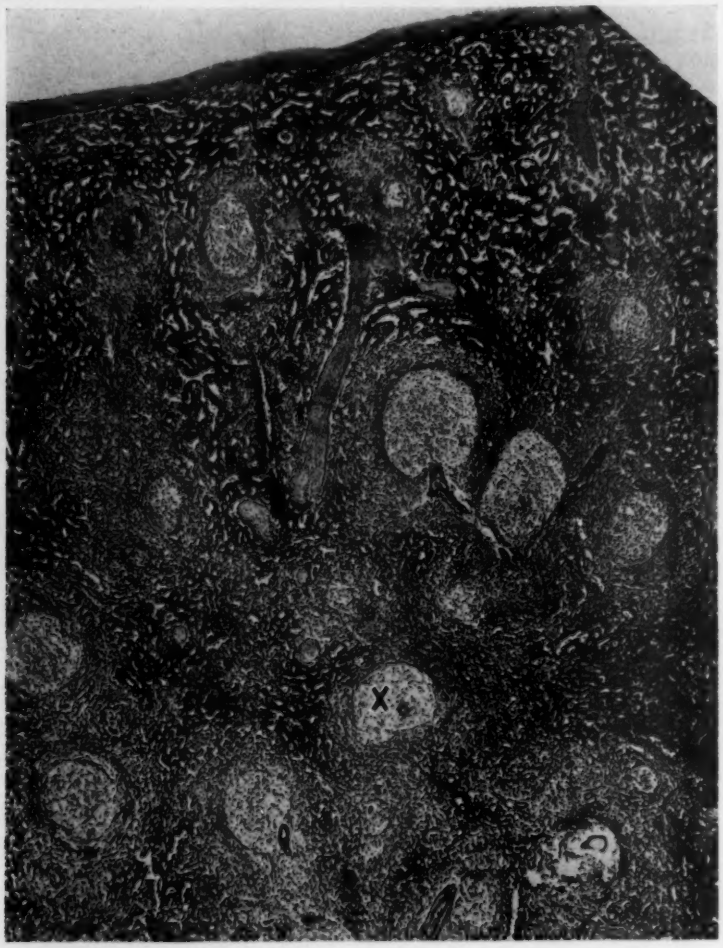

Fig. 4.—Peripheral portion of a vertical section of the spleen. The white pulp is of relatively great bulk. The red pulp is more extensive and conspicuous in the subcapsular region. The white pulp contains numerous rarefied nodules, consisting of a loose and discontinuous stroma of reticular tissue with a sparse parenchyma of scattered small lymphocytes. Each nodule is delimited by a narrow corona of small lymphocytes. Helly fixation; eosin-azure stain; magnification, 40 diameters.

sinuses. Similar areas showed sinuses filled with erythroblasts and normoblasts, except for a peripheral border of larger lymphocytes. The

intervascular stroma consisted of more scattered lymphocytes intermingled with maturing eosinophils and basophils. Both areas sometimes contained scattered plasma cells and an occasional Russell body. The histologic observations suggest that erythropoiesis is restricted to the venous sinuses and granulopoiesis to the intervascular stroma, the large

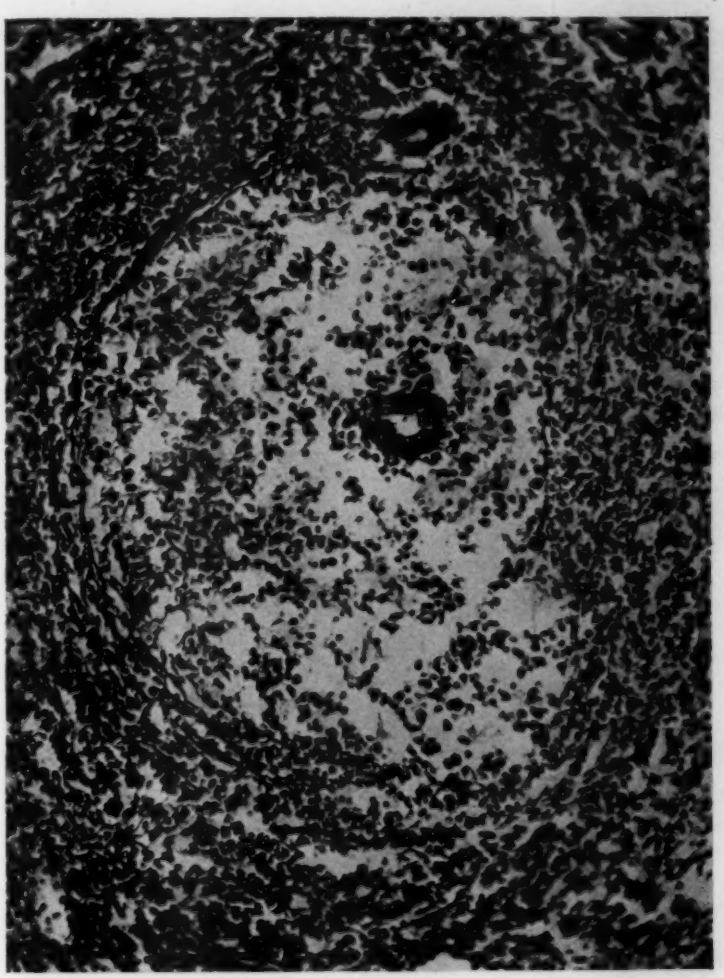

Fig. 5.—Nodule ("Malpighian corpuscle," *X* of fig. 4). The nodule contains a subcentral arteriole. Such "nodules" apparently represent germinal centers that are now exhausted and almost completely drained of lymphocytes. This nodule contains no macrophages. Helly fixation; eosin-azure stain; magnification, 300 diameters.

lymphocytes serving in both regions as ancestral cells. The meager mitosis was limited to larger lymphocytes (hemocytoblasts) and erythro-

cytes. This marrow was characterized further by the presence of considerable numbers of small, pale normoblasts with fragmenting nuclei, obviously in various stages of degeneration. The general condition of the marrow was one of hypoplasia, not aplasia. No areas of fibrosis were seen.

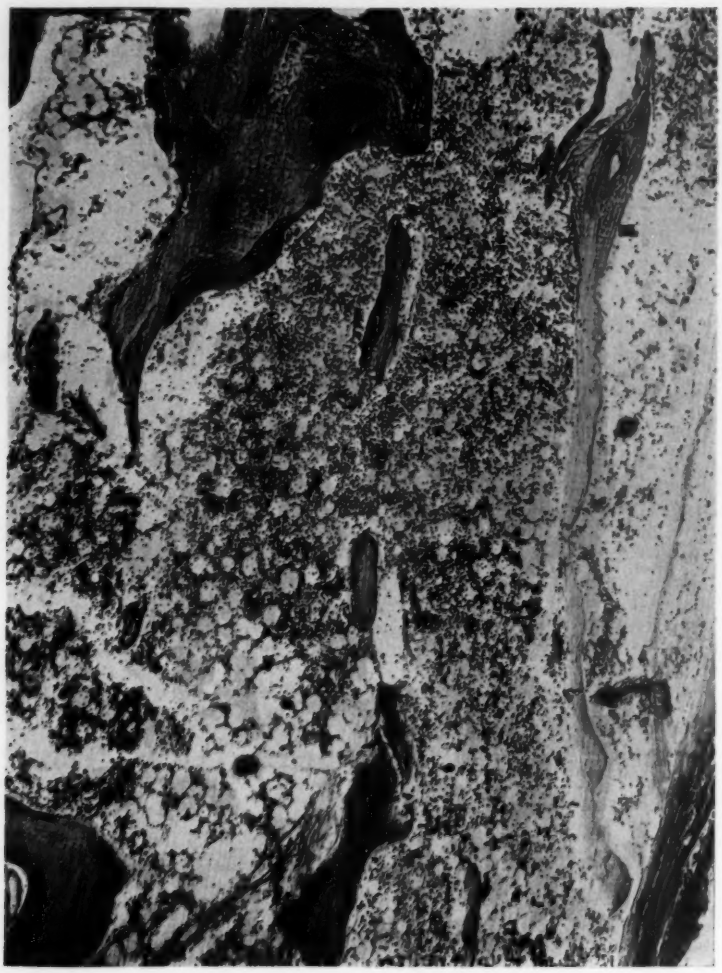

Fig. 6.—Marrow of a rib. The central area represents the average, moderately diffuse condition. Helly fixation, eosin-azure stain; magnification, 40 diameters.

In sections stained with eosin-azure the large lymphocyte (hemocytoblast) had deeply basophilic (blue) cytoplasm. The relatively large nucleus was vesicular and practically achromatic (fig. 3 *a*). It generally contained one or several larger spherical or oval plastin nucleoli, which

stained red, and several smaller, frequently irregular chromatic (blue) granules. There might be in addition several short pale delicate strands of linin and some minute pale granules. The nuclear membrane was generally very delicate but conspicuous. The nuclear membrane showed

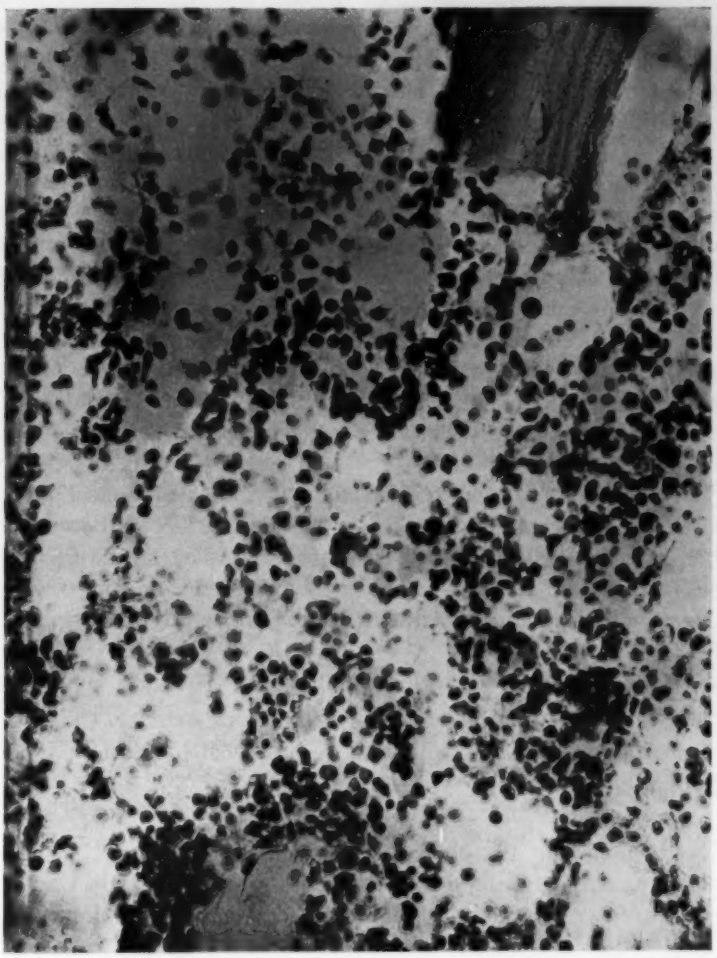

Fig. 7.—Area from center of figure 6. The predominating cell is a small lymphocyte. Helly fixation; eosin-azure stain; magnification, 300 diameters.

considerable variation in the degree of delicacy. The cytologic observations on the medium-sized lymphocyte were essentially identical. The small lymphocyte differed slightly in that the narrow cytosome stained a somewhat lighter shade of blue, the nucleus generally had only one plastin nucleolus and the chromatin granules were somewhat coarser

and more conspicuous. This description applies equally well to the lymphocytes of the lymph nodes and spleen.

Basophilic granulocytes occurred in relatively larger numbers, perhaps slightly more frequently than eosinophils. Basophils and eosinophils had the same variations of size, the same type of nucleus and spherical granules of the same size. However, two varieties of basophils occurred: one with blue staining granules and one with purple granules of varying depth of shade. The majority were of the latter type. Some of the latter variety appeared to be in process of degeneration, showing only scattered granules. The evidence here accords with the concept of basophils as unripe (immature) and abortive eosinophils.

An unexpected observation was the apparent total absence of megakaryocytes. This seemed so unusual that a special search was made for these cells. Typical megakaryocytes could not be found. There were not even recognizable nuclear remains of such cells. These cells must certainly have been scarce. Several small pale-staining polykaryocytes were found; these were more probably atrophic osteoclasts. One may suggest that the available hemocytoblasts were so urgently needed for the production of erythrocytes that none could be spared for the production of megakaryocytes.

The general appearance of the marrow indicated almost complete exhaustion of the essential factors for the maturation of the decreasing number of available hemocytoblasts into erythrocytes. Moreover, all of the hemopoietic tissues—lymph nodes, spleen and marrow—gave histologic indications also of exhaustion of proliferative ability.

LIVER

The liver showed no indication of hemopoietic activity. The hepatic cells contained considerable amounts of yellowish pigment in the form of granules and flakes.

COMMENT

The hemopoietic tissues in this case of "aplastic" (hypoplastic) anemia, i. e., the lymph nodes, spleen and bone marrow, had an essentially uniform, predominantly lymphoid character. These tissues should be considered as an organic unit. All showed consistent hypoplasia and gave evidence of functional exhaustion. The dominating cell was one with the essential features of a small lymphocyte. This cell grew and acquired the features of a typical hemocytoblast. In the bone marrow the hemocytoblasts within venous sinuses differentiated into erythroblasts. Identical hemocytoblasts in the intervascular stroma differentiated into eosinophils, basophils and neutrophils. The primary object of this study, as stated, was to apply the concept of the small lymphocyte as an embryonal cell with multiple developmental potencies, including

the capacity to serve as an ancestor for erythrocytes, in an interpretation of the histologic conditions in the hemopoietic tissues.

The most striking histologic feature of this case was presented by the spleen. Though of approximately normal size, the nodules were greatly increased in number. Moreover, these nodules had in general large rarefied centers, consisting of a loose and largely discontinuous reticulum with scattered lymphocytes. No nodule with a germinal center occurred. Lymphocytes of the large variety were relatively rare. Proliferating lymphocytes were extremely rare.

Some of the nodules contained a considerable number of degenerating and disintegrating lymphocytes; a few had fibrotic central areas; a few contained large pale cells with the features of macrophages. However, these macrophages contained practically no débris. The sinuses were relatively wide and contained much blood. The appearance of the spleen suggested the end phase of a long period of increased lymphopoietic activity. This interpretation was based on the presence of a greatly increased number of nodules, their exhausted histologic condition and the absence of phagocytic activity, though considerable débris was occasionally available. These conditions seem to time precisely the spleen's lymphopoietic activity at a point of almost complete exhaustion.

The predominant tissue of the marrow was lymphoid. Erythropoiesis and granulopoiesis were at low ebb. The lymphocytes of the marrow were essentially exact duplicates of those of the spleen and lymph nodes. Though the reticular stroma of the marrow was a potential source of lymphocytes (hemocytoblasts), its proliferative inactivity and its relatively very meager occurrence suggested that the lymphocytes had at least largely an extraneous origin. The appearance of the lymph nodes and especially that of the spleen suggested that these tissues functioned at forced capacity to furnish lymphocytes to the marrow for source material for erythrocytes and granulocytes. If this interpretation as applied to this case has the merit that is suggested by the results of a comparative study of blood-forming tissues of submammalian vertebrates,[4] the data obtained in this case add considerable support to the explanation for the apparent daily disappearance of an enormous number of lymphocytes: They are filtered out in the bone marrow, where they furnish at least a considerable proportion of the mother cells of erythrocytes.

Opponents of the unitarian interpretation of the development of blood stress certain alleged minute structural differences between lymphoblasts of lymph nodes and spleen and myeloblasts of bone marrow. Some[5] claim ability to discriminate two or three varieties

4. Jordan, H. E.: Quart. Rev. Biol. **8**:58, 1933.

5. Piney, A.: Recent Advances in Haematology, Philadelphia, P. Blakiston's Son & Co., 1927. Cunningham, R. S.; Sabin, F. R., and Doan, C. A.: Contrib. Embryol. **16**:227, 1925.

among the myeloblasts: granuloblasts, the ancestors of eosinophils, basophils and neutrophils; proerythroblasts ("megaloblasts"), the ancestors of erythrocytes; monoblasts, the ancestors of monocytes. The differential features concern degree of delicacy of nuclear membrane, number of nucleoli, abundance and manner of distribution of nuclear chromatin, character and number of mitochondria and of neutral red bodies and azurophilic granules, and depth of basophil staining of the cytosome. It is possible on the basis of these criteria to select a lymphoblast in the spleen or in a lymph node which differs from a myeloblast of the marrow; it is further possible to select among myeloblasts, ancestral cells of granulocytes, monocytes and erythrocytes which appear to show differential features. But to emphasize such differences cells must be *selected,* and variations within each group must be *ignored.* And the criterion employed by some to judge the specific character of these ancestral cells, namely, the "company which they keep," must be recognized for what it actually is: an evasion of the fundamental issue, an interpretation without morphologic or cytologic validity.

When attention is directed to variations within each group alleged to be specific, so-called lymphoblasts, proerythroblasts, granuloblasts and monoblasts, one finds that the variations within each group are as wide as regards the alleged differential criteria as the differences between the groups and that these variations in each group overlap those among the groups. In view of these facts the logical position seems to be one which recognizes a common ancestral cell for erythrocytes, granulocytes, lymphocytes and monocytes and emphasizes the well established designation "hemocytoblast" for this cell. Accordingly, lymphoblasts are actually hemocytoblasts resident in lymph nodes and spleen, proerythroblasts are the hemocytoblasts in bone marrow which differentiate into erythrocytes, and myeloblasts are hemocytoblasts which differentiate into granulocytes. The hemocytoblasts are common blood mother cells, relatively undifferentiated, with multiple developmental potencies, the developmental bias resulting from a specific differential environmental stimulus. And finally the evidence indicates that the so-called small lymphocytes are essentially small hemocytoblasts with all the developmental capacities of primitive cells. The recent studies of Bloom [6] and Conway [7] seem to show conclusively that in experimentally increased monocytopoiesis the small lymphocyte constitutes a hemocytoblast which as a "monoblast" produces the monocyte.

Little significance can be attached to the occurrence of normoblasts in the spleen since some were found in the blood stream. The normoblasts of the spleen were certainly at least in part carried there from the marrow. There is some evidence that the spleen functioned at least

6. Bloom, W.: Arch. Path. **6**:995, 1928.
7. Conway, E. A.: Arch. Path. **25**:200, 1938.

to a slight degree as an erythropoietic organ, but at the time of death such function was negligible as compensatory to the hypoplasia of the marrow.

The occurrence of considerable numbers of plasma cells in the spleen and marrow is probably significant. I have previously supported the concept of the plasma cell as an abortive erythroblast.[8] The plasma cell passes through maturation stages comparable with those of the erythroblast; the essential difference is an absence of hemoglobin and the presence of a juxtanuclear achromatic oval area or vacuole in the plasma cell. The plasma cell represents a modified lymphocyte; the nuclear changes in its life cycle appear identical with those of the erythroblast and end with enucleation and the formation of a plastid. The concomitant occurrence of a relatively small chromatic eccentric nucleus with a juxtanuclear achromatic area or vacuole in the plasma cell signifies a reciprocal relationship: As the nucleus contracts and becomes more chromatic, the expressed nuclear sap collects in the juxtanuclear area and dilutes the staining capacity of this region while pressing the nucleus eccentrally. The erythroblast plastid (red blood corpuscle) is hemoglobiniferous; the plasma cell plastid is polychromatophilic and practically ahemoglobiniferous. If the concept of the plasma cell as an abortive erythroblast has validity, the occurrence of plasma cells under conditions approaching erythropoietic exhaustion may signify a phase of the unsuccessful effort to combat an anemia.

The occurrence of relatively large numbers of basophilic granulocytes in the marrow and lymph nodes calls for explanation. Some of these granulocytes, especially those caught in extended ameboid activity, suggested mast cells of subcutaneous tissue. This suggestion was emphasized by the fact that only relatively few had blue granules after eosin-azure staining while the majority had purple or lilac granules. However, there is no significant variation in size, nor is there any difference of nuclear character, among these tinctorial and morphologic varieties. Furthermore, they are within the limits of variation in size among the associated eosinophils. In earlier publications[9] I supported the concept of the basophils as either immature or abortive eosinophils. The basophils with blue-staining granules are almost certainly unripe eosinophils; those with purple granules may be regarded as abortive eosinophils. In the present case many of the basophils with purple granules showed degenerative features as regards the nucleus and dispersal and irregularity of the granules. One seems to be justified in concluding that in this case of aplastic anemia the conditions which underlie the inability of the reduced supply of available hemocytoblasts

8. Jordan, H. E.: Anat. Rec. **42**:91, 1929. Jordan, H. E., and Morton, C. B.: Am. J. Anat. **61**:407, 1937.

9. Jordan, H. E.: Am. J. Anat. **51**:215, 1932; Anat. Rec. **71**:102, 1938.

to differentiate into erythrocytes, resulting in part in the production of plasma cells, operated also in the production of abortive eosinophils, the cells with purple-staining granules.

From the point of view of the special object of this study of a case of aplastic anemia the central fact concerns the fairly clear evidence that the lymph nodes and especially the spleen functioned with increased activity to supply lymphocytes to the bone marrow to serve as ancestors for erythrocytes. Apparently the bone marrow became unable both to produce hemocytoblasts in normal numbers and to effect differentiation at a normal tempo, so that the spleen especially was stimulated to compensatory function. The final appearance was one of almost complete exhaustion of lymph nodes, spleen and bone marrow. Since under these conditions the bone marrow apparently became almost wholly dependent on an extraneous supply of lymphocytes from lymph nodes and spleen, it seems logical to suggest that under normal conditions also the bone marrow utilizes lymphocytes filtered from the blood for the normal production of erythrocytes. Such utilization would give an adequate explanation of the daily "loss" from the body of an enormous number of lymphocytes aggregating approximately two and one-half times the number normally present in the blood stream.[10]

SUMMARY

An effort has been made to apply the concept of the lymphocyte as an immature polyvalent cell in an interpretation of the histologic observations on the hemopoietic tissues of a patient who died with clinically typical "aplastic" hypoplastic anemia. The lymph nodes, spleen and bone marrow were considered as a unit. These three tissues had essentially the same lymphoid structure. They had an essentially uniform appearance of hypoplasia. The lymph nodes and spleen showed evidence of almost complete exhaustion following a period of excessive activity. The predominating cell in the three tissues was the small lymphocyte. Large and medium-sized lymphocytes were relatively more abundant in the bone marrow. The small lymphocytes grew to become large lymphocytes which as hemocytoblasts differentiated into erythrocytes within the venous sinuses and into granulocytes in the intervascular stroma. The evidence is in complete accord with the claim that at least one important function of the lymphocytes is to serve as ancestors for erythrocytes and granulocytes. The marrow practically lacked megakaryocytes. It contained many basophilic granulocytes, interpretable as abortive eosinophils. Both spleen and marrow contained considerable numbers of plasma cells, interpretable as abortive erythroblasts. and many small, pale, disintegrating normoblasts.

10. Yoffey, J. M.: J. Anat. **67**:250, 1933.

MEMBRANE FORMATION AT LIPOID-AQUEOUS INTERFACES IN TISSUES

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Histologic studies have shown that several liquid soluble oils and their derivatives are partly transformed into semisolid insoluble substances during a period of two or three weeks' residence in the subcutaneous tissues of guinea pigs.[1] Chemical compounds amenable to this change of state have at least two characteristics in common. First, they have long normal hydrocarbon chains terminated with a free or esterified carboxyl group. Second, the hydrocarbon chains have at least two unconjugated ethylenic linkages. A larger number of double bonds and binding of the chains through ester linkages with glycerol seemed to favor the transformation. The conclusion was drawn that addition of oxygen at the double bonds and subsequent polymerization of some products of partial oxidation are fundamental steps leading to the formation of the insoluble materials.

These materials were in the form of granules, globules and membranes. Their variable physical properties were a function of the compounds introduced into the tissues. The continuous membranes most satisfactory for study developed at the lipoid-aqueous interface of residual globules of highly unsaturated compounds and the limiting formed or fluid tissues. This report is concerned with a study of these membranes.

MATERIALS AND METHODS

Previous studies indicated that cod liver oil and methyl esters of unsaturated acids of cod liver oil were satisfactory sources of materials. With the hypothesis in mind that intercellular oxygenation is one step in the process of transformation in vivo, methyl esters of unsaturated acids of cod liver oil were oxygenated in vitro and used as the third source of compounds.

Medicinal cod liver oil from which free fatty acids had been removed was used. Methyl esters of unsaturated acids of cod liver oil were prepared by methods described elsewhere. The total fraction of the methyl esters (boiling point from 145 to 205 C. at about a pressure of 1 mm. of mercury) was divided into two parts. One part was stored in the refrigerator under carbon dioxide. The remainder, 25 cc., was placed in a small flask. To this was added 5 mg. of ferrous sulfate. Atmospheric oxygen was bubbled through the solution for five days at room temperature. The esters, initially almost colorless, became brownish yellow, and their viscosity increased slightly. After the five day period of oxygenation,

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1. Hass, G. M.: Arch. Path. **26**:1196 (Dec.) 1938.

the esters were dissolved in ether and washed thoroughly with dilute potassium hydroxide. The excess of alkali was removed from the ethereal solution by repeated washing with water. The ether and the more highly volatile products of oxygenation were removed by vacuum distillation. The residual oxygenated esters formed a clear yellow liquid which was less viscous than cod liver oil. The oxygenated esters were placed in the dark at room temperature for three weeks. During this time a thin yellowish film formed on the wall of the flask. The liquid esters, easily soluble in ether, were removed. The film resisted the solvent action of ether boiled in the flask for forty-eight hours under a reflux condenser. The film was removed mechanically. It was pliable and somewhat resilient, although friable. On drying it formed a yellowish brittle amorphous powder.

Acid-free cod liver oil, liquid methyl esters of unsaturated acids of cod liver oil preserved under carbon dioxide, and liquid oxygenated methyl esters of unsaturated acids of cod liver oil were injected subcutaneously into the abdominal walls of young guinea pigs in doses of from 0.05 to 0.1 cc. The animals were put to death at the end of two, three and four weeks. Segments of their abdominal walls including the sites of injection were fixed for twenty-four hours in solution of formaldehyde U. S. P. diluted 1 : 10. Representative blocks of tissue were dehydrated in graded alcohols and embedded in paraffin. Paraffin sections were prepared with the following stains: hematoxylin and eosin; Ziehl-Neelsen stain for the demonstration of acid-fast micro-organisms; Weigert's stain; Verhoeff's stain; Unna's orcein stain for demonstrating elastic tissue.

A few special studies were made as follows: In order to establish comparative staining reactions of the solid material obtained by oxygenation of the esters in vitro, some of the amorphous powder, a few milligrams, was injected subcutaneously into guinea pigs. The animals were put to death three days later and the tissues treated by the routine methods described.

Paraffin sections were placed in various concentrations of neutral aqueous potassium permanganate (0.01 to 0.1 per cent) at room temperature for periods of from one to three hours. The effect of chromate oxidation was studied by immersing paraffin sections in Regaud's fixative for from twenty-four to forty-eight hours before application of the routine stains. Paraffin sections, as well as blocks of tissue fixed in solution of formaldehyde, were used in determining the solubility of the membranes in ether and chloroform. Paraffin sections were satisfactory for preliminary observations on the resistance of the membranes to the action of trypsin at p_H 8 to 8.5 and at from 35 to 37 C.

EXPERIMENTAL RESULTS

Methyl esters of unsaturated acids of cod liver oil, preserved under carbon dioxide, did not yield long continuous membranes during an interval of two, three or four weeks in the subcutaneous tissues. They caused local necrosis of tissues and were partially transformed into granules, globules and shreds of insoluble material.

Acid-free cod liver oil and liquid soluble oxygenated methyl esters of unsaturated acids of cod liver oil were partly transformed into long continuous membranes at the lipoid-aqueous interface of the residual injected material and the tissues or tissue fluids. In addition, they were

changed into granules and globules of insoluble material which resided either in the tissue spaces or in the cytoplasm of macrophages and giant cells. Several differences existed between membranes which formed in the presence of cod liver oil and those which appeared at the interface of oxygenated methyl esters and the tissues. For the purposes of this presentation, the membranes may be considered as essentially similar.

PHYSICAL PROPERTIES OF THE MEMBRANES

The membranes are usually long and continuous, occupying the zone of contact between residual liquid lipoidal materials and cell margins or intercellular tissues. When in contact with the margins of fibroblasts and macrophages, they often seem to be continuous with the protoplasm of the cell membrane. When in apposition to condensed strands of fibrin or collagenous fibrils, the zone of contact is usually distinguishable, although at times fibrin and collagen are incorporated in the membranes. In general, permeation of the lipoids into the aqueous phase containing various intercellular structures is greatly restricted.

The membranes vary in thickness from delicate structures which are just within the resolving power of the microscope to approximately 10 microns. There appears to be an upper limit of thickness (about 10 microns) which is never reached by the structures arising from oxygenated methyl esters and which is not readily exceeded by the membranes which form in the presence of the oil.

The membranes are usually wavy in outline and thrown into folds, although they are occasionally straight or curved in conformance with the linear or curved surface of the lipoid-aqueous boundary. They are almost colorless but may be detected in some sections by a light yellowish tint which they possess. They are highly refractile and usually appear to be optically homogeneous. In stained preparations it is possible to detect in a few instances a reticular, vacuolated or fibrillar structure.

As a rule the margin of the membrane bordering on the aqueous phase is well defined. The margin of the membrane bordering on the lipoidal phase is usually distinct but not always sharp in outline.

When disrupted they fracture transversely, undergo flexion and assume a wavy contour or disintegrate into granules. They are rather pliable than rigid and have a certain tenacity by which large segments retain their continuity even though they have been displaced by the granulation tissue from the zone at which they formed.

Their behavior indicates that they have a higher elasticity, in the common meaning of the term, than the tissue elements with which they are in contact. The wavy contour of many membranes is comparable with that of the internal elastic membranes of medium-sized arteries, included in the same section.

CHEMICAL PROPERTIES

The membranes are insoluble in water, alcohol, xylene, ether and chloroform. They are acidic, as indicated by their affinity for basic fuchsin. They often retain phenolized fuchsin, resisting decolorization

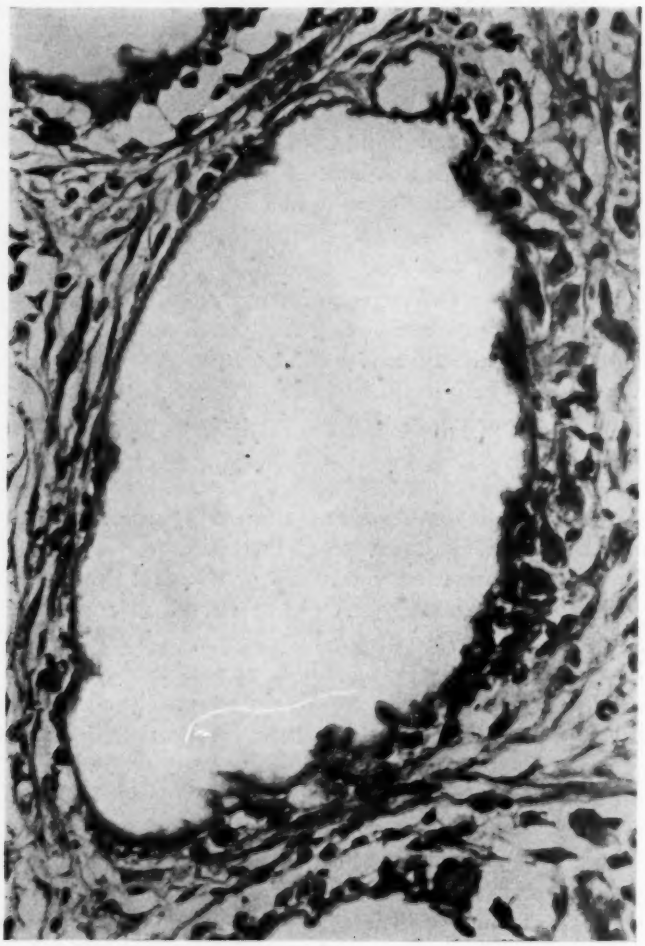

Fig. 1.—Low power photomicrograph which shows the position of the membrane at the lipoid-aqueous tissue interface. The clear area in this section was occupied by an oil globule. The narrow membrane is shown at the junction of the oil globule and the connective tissue. Material, acid-free cod liver oil; Verhoeff's stain for elastic tissue.

quite as effectively in many instances as does the tubercle bacillus when treated by the same methods. The acid-fast property is variable but is more pronounced when cod liver oil is utilized as the source material.

It is acquired slowly, reaching a maximum at three weeks, and then either remains constant or diminishes. With the diminution of the acid-fast property, an affinity for the methylene blue counterstain is often acquired.

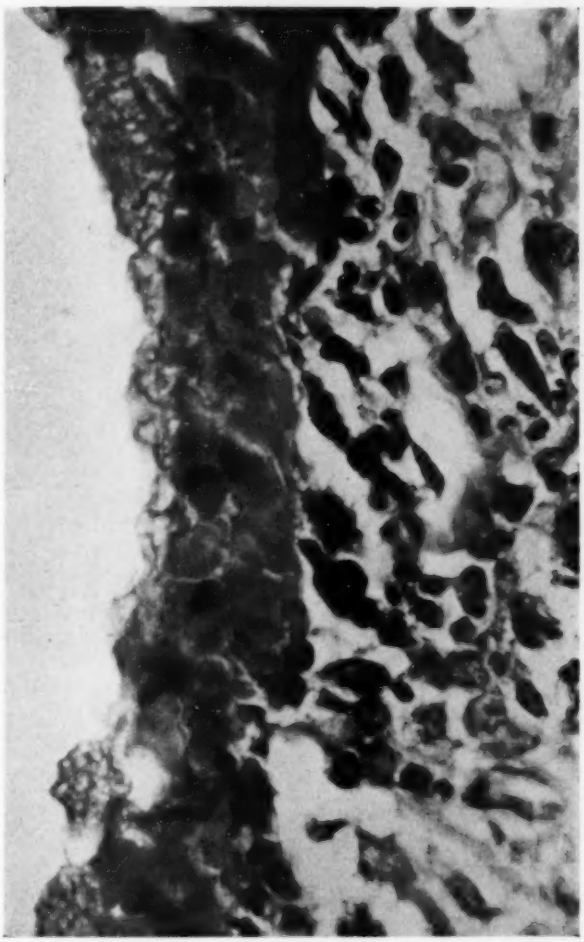

Fig. 2.—High power photomicrograph which illustrates the rarely observed reticular character of the membrane. The clear area to the left of the membrane is the site of the soluble lipoid globule. The aqueous phase at the right of the membrane is largely occupied in this region by degenerating red blood cells in granulation tissue. The reticulum of the membrane stains similarly to elastic tissue. Material, oxygenated methyl esters of unsaturated acids of cod liver oil; Verhoeff's stain for elastic tissue.

Weigert's resorcinol-fuchsin stain for demonstrating elastic tissue discloses not only the acidity but also the reducing power possessed

by the membranes. These properties are readily detectable at two weeks and are more clearly defined at the end of three or four weeks. At this time the membranes have achieved the full depth of staining by the resorcinol-fuchsin method and bear a close resemblance to the elastic

Fig. 3.—High power photomicrograph which illustrates the characteristic homogeneous wavy condensed membrane. It is present on both aspects of collagenous trabeculae which pass between four lipoid globules represented by the clear areas at the left and right margins of the photograph. Material, oxygenated methyl esters of unsaturated acids of cod liver oil; Weigert's stain for elastic tissue.

membranes of arteries and veins included in the same section. The positive staining by the resorcinol-fuchsin and that by the phenolized fuchsin method are often related in intensity. This is not always true, especially with membranes arising in the presence of oxygenated methyl

esters. These membranes at three and four weeks usually stain deeply with resorcinol-fuchsin and very lightly with phenolized fuchsin.

The application of Verhoeff's and Unna's stains for elastic tissue gives results which are comparable with those obtained by Weigert's

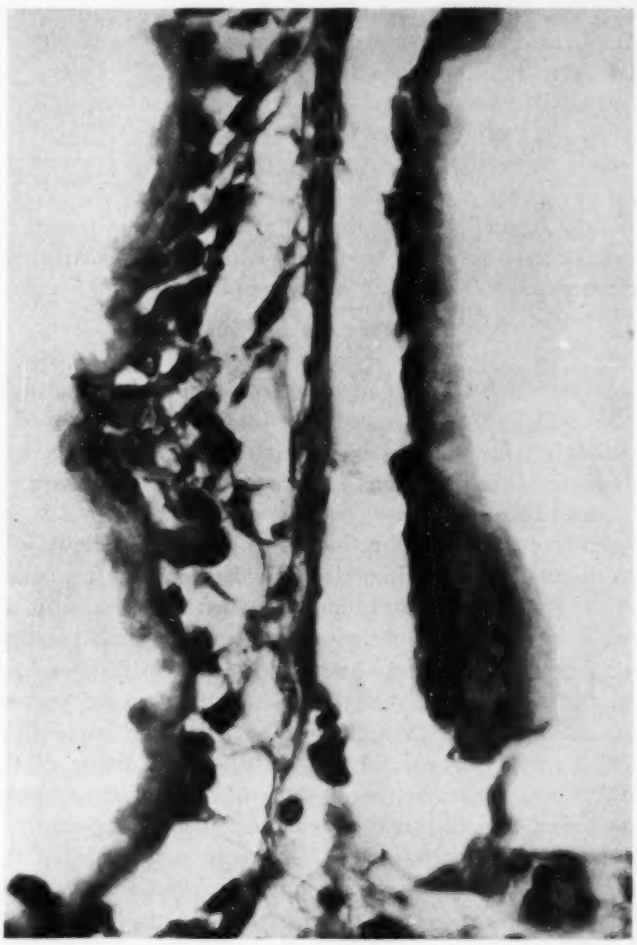

Fig. 4.—High power photomicrograph illustrating typical membranes in the early stages of their formation. Positions of lipoid globules are at the left and right margins of the photograph. Note the continuity of each membrane with margins of limiting cells and their extension from the lipoid-aqueous interface in each instance into the lipoidal phase. Material, acid-free cod liver oil; Verhoeff's stain for elastic tissue.

method. Unna's stain is less satisfactory than Verhoeff's stain. By Verhoeff's method the mature membranes stain bluish purple and are sharply defined.

That the acid-fast property and intense staining by resorcinol-fuchsin are potential properties of unsaturated methyl esters transformed in vivo is well shown by the election of carbolfuchsin and resorcinol-fuchsin stains by the solid substances derived from oxygenation of the esters in vitro.

The membranes are more susceptible to oxidation by neutral aqueous potassium permanganate than the regional collagenous tissues. During mild oxidation they acquire a delicate brownish tinge, which is accentuated at the two surfaces. Treatment with potassium dichromate also discolors the membranes and in the early stages of their formation increases their susceptibility to staining by Weigert's resorcinol-fuchsin method.

When fully formed, the membranes are resistant to tryptic digestion and are usually the only intact structures which can be clearly recognized in sections exposed to the action of trypsin at p_H 8 to 8.5 for from six to twelve hours at 37.5 C.

COMMENT

Membranes which form at the interface of acid-free cod liver oil or oxygenated methyl esters of unsaturated acids of cod liver oil and the cells or fluids of the subcutaneous tissues of guinea pigs have been described. The cellular reactions which accompany the presence of these structures have been considered elsewhere. Suffice it to say that giant cells frequently encapsulate the thick membranes, especially when they have been disrupted and dislocated from the zone of formation. This cellular reaction is much more prominent when cod liver oil is employed and is usually inconspicuous in the presence of oxygenated methyl esters.

Some of the physical and chemical properties of these membranes have been presented here. Often these properties are such that the continuous structures are morphologically indistinguishable from membranes which are a part of the system of elastic tissue of the body. Several characteristics are similar to those of some basement membranes.

Elastic tissue and basement membranes consist primarily of proteins, although several investigators have expressed the belief that other unknown elements enter into their composition.

There are no data at present which confirm a suspicion that materials other than lipoids are concerned in the formation of the structures described here. Although fibrils may occasionally be distinguished in the membranes or ensheathed by the membranes, the extent to which available proteins in the aqueous phase are implicated cannot be accurately determined by the methods employed in these experiments. Furthermore, the systems which have been described are as yet too complex to yield to an analysis based on ideas of adsorption, orientation of polar groups or chemical binding. The fundamental reaction occurs

at the lipoid-aqueous interface and proceeds to a limit into the lipoidal rather than into the aqueous phase.

The limit is quite uniform for each lipoid mixture employed. For any given globule the membrane over the entire boundary, which is often several square millimeters in area, does not vary in thickness more than 1 or 2 microns from a constant value. This constant value is greater when the oil is employed, membranes around residual globules of methyl esters being much more delicate. This value is retained in each instance despite prolongation of the time of residence of the compounds in the tissues. If oxygen or oxygen donators are implicated in the mechanism of transformation, these limits mark the zone of their effective movement in the lipoidal phase. It is possible that by an appropriate selection of materials, increased permeation of the aqueous phase by lipoids or increased diffusion into the lipoidal phase of intercellular materials may occur at the interface. A third zone, essentially lipoprotein in composition, may thus be available as a model for study.

There is no proof that the membranes described here are of biologic significance. Reasons for believing that they may have significance are threefold: First, they resemble some membranes which are formed in the animal from materials and by mechanisms which are unknown. Second, the types of compounds which give origin to the membranes are representative of biologic materials which are tolerated and transported with regularity in the circulating tissues and in the protoplasm of cells of the body. They occur uncombined as acids or in combined forms, such as glycerides, cholesterol esters, phosphatides and other compounds. Third, the transformation of the unsaturated compounds occurs under conditions in tissues which are not wholly unphysiologic.

The resolution of one aspect of the problem to mixed products of oxygenated methyl esters of highly unsaturated fatty acids is desirable from the standpoint of possible identification of materials which exhibit this unusual behavior at a lipoid-aqueous tissue interface.

Polymerization of dextrose and of aminoacids is a well established process in the mammalian body. It is perhaps justifiable to think along the lines that polymerization of some unsaturated acids or compounds of which they are a part through activation of the ethylenic linkages and creation of polar groups by partial oxidation may also occur and contribute to the construction of some bodily tissues.

SUMMARY

Acid-free cod liver oil, methyl esters of unsaturated acids of cod liver oil and oxygenated methyl esters of unsaturated acids of cod liver oil were injected into the subcutaneous tissues of guinea pigs. The persistence of these compounds or their derivatives in situ permitted

the study of a lipoid-aqueous interface under physiologic conditions in vivo. When acid-free cod liver oil and oxygenated methyl esters of unsaturated acids of cod liver oil were employed, distinct membranes formed at the interface. These were limited on one aspect by the lipoid from which they were principally derived and on the other by the margins of cells, fibrin strands, collagenous fibrils and intercellular fluids. The membranes developed gradually and when fully formed possessed several properties which were similar to those of elastic tissue and some basement membranes of the mammalian body.

ARGENTAFFIN (CARCINOID) TUMORS OF THE SMALL INTESTINE

REPORT OF ELEVEN CASES AND REVIEW OF THE LITERATURE

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Carcinoid (argentaffin) tumors have been the subject of much discussion and interest since they were first differentiated from carcinoma by Oberndorfer in 1907. The first authentic report of cases of carcinoid tumors of the small intestine, however, is attributed to Lubarsch. This author in 1888 reported 2 cases of tumors of the small intestine, which he termed primary carcinomas. These were multiple, small, nonmetastasizing tumors, consisting of masses of cells that did not assume glandular arrangements and bore no similarity to the normal intestinal mucosa. In 1907 Oberndorfer, on the basis of morphologic studies, advocated the separation of these neoplasms from true carcinomas and suggested the term "carcinoid" to emphasize their benign nature.

The histogenesis of carcinoids has been a perplexing problem, and attempts at its elucidation have resulted in many theories regarding their origin. Trappe believed that both carcinoids and adenomyomas originated from the pancreatic rests that are commonly found in the gastrointestinal tract. Oberndorfer also favored this view. Burckhardt considered them identical with basal cell carcinomas of the skin. Toenniessen felt that carcinoids originated from submucous rests representing glands of internal secretion and were in that sense similar to the islands of Langerhans. Huebschmann believed that carcinoids arose from the *gelben Zellen* of the intestinal mucosa, which represent the cells originally described by Kultschitzky and which later were called argentaffin cells by Masson and Gosset. Saltykow was of the opinion that they arose from pancreatic rests which produced only islet tissue, while adenomyomas arose from pancreatic rests which gave rise exclusively to glandular tissue. Ehrlich believed that they arose from Auerbach's plexus and called them "immature sympathetic neurocytomata." Krompecher agreed with Burckhardt that these tumors were identical with basal cell carcinomas of the skin. Aschoff thought that they represented nevi of mucous membrane, and Danisch considered them of celiac ganglion origin. Engel and Primrose believed that carcinoids arose from embryonal rests. The former called them "choristblastoma" and the latter "embryoma."

It was not until the work of Masson that the histogenesis of carcinoids was more fully explained. This author, on the basis of special histologic technic, showed that these neoplasms were derived from the chromaffin cells of the crypts of Lieberkühn and suggested the term "argentaffine tumor" because of the ability of these cells to reduce solutions of ammonical silver. He further designated these new growths as endocrine tumors because he believed that the chromargentaffin cells of the intestine constitute a diffuse endocrine organ.

From the laboratories of the Mount Sinai Hospital.

In 1928 Masson suggested that the secretions of the Kultschitzky cells act on the mucosal plexus, influencing the activity of the muscularis mucosa, and therefore he suggested the term "neurocrine tumor." In 1930 he stated that because carcinoids originate in previously hypertrophied nerves of the mucosa and progressively penetrate the myenteric plexus, without destroying or causing it to become hyperplastic, these new growths merit the name "neurocarcinoids." He believed in the existence of a neurentoderm, an entodermic placode, of which the cells of Kultschitzky are the sole cellular manifestation in normal conditions. However, the precise nature and function of these cells have not been satisfactorily determined.

The various views concerning the origin of carcinoids may be summarized as follows:

1. They are forms of true carcinoma arising from the gastrointestinal epithelium.
2. They are identical with basal cell carcinoma of the skin.
3. They represent growths from embryonal rests.
4. They have their origin in the sympathetic nervous system.
5. They arise from the argentaffin cells of the intestinal epithelium.

The last view, advocated by Masson, is now generally accepted.

The benign nature of these tumors has been stressed by several authors (Oberndorfer; Saltykow; Forbus). Although their tendency to infiltrate has long been known, it has only recently been recognized that they may produce clinical symptoms and assume all the properties of malignant growths. At present their differentiation from carcinoma is not generally appreciated. Thus Rankin and Mayo in a report of carcinoma of the small intestine made the following statement:

> Great difficulty was met in reviewing the literature. The main problem lay in detecting cases in which carcinoid tumors were wrongly classified under the title carcinoma. This fault occurred all too frequently and the effort to glean the actual cases of carcinoma was doubted.

Carcinoid tumors of the small intestine are still considered by many as benign, insignificant pathologic curiosities.

The purpose of this study is to present 11 carcinoid tumors of the small intestine. Four of these had produced symptoms of intestinal obstruction which necessitated operative intervention. Metastases were found in 3 of the cases in which obstruction occurred and in 2 other instances. Five examples of purely benign growths are also presented. Ten of these were observed in a series of 47,045 specimens (both autopsy and surgical material). In the same series 21 appendical carcinoids were found. The eleventh tumor was one observed by Drs. J. Ehrlich, M. Bookman and M. Cohen, of the Lebanon Hospital, New York.

REPORT OF CASES

Case 1.—A 66 year old Negress had undergone a hysterectomy in 1914 for what was probably an ovarian tumor. Two months before admission she was

suddenly awakened from her sleep by a severe colicky epigastric pain. Some hours after the onset she took several doses of cathartics but vomited after each dose. The following day she had loose bowel movements and passed much flatus. At this time she complained of soreness and pain in the right lower quadrant of the abdomen. The pain had recurred frequently and had usually been postprandial, starting in the epigastrium and shifting to the right lower quadrant. Three months before admission she had noted tarry stools, which had recurred frequently. Two months prior to admission a mass in the right lower quadrant was associated with the pain. She had not complained of nausea or vomiting since the first episode, at the onset of the illness. Fever was not present. She had lost 33 pounds (15 Kg.), had felt weak since the onset and two days before admission experienced chilly sensations.

On admission to the hospital, April 12, 1935, she presented an irregular, fixed, ill defined tender mass in the right lower quadrant of the abdomen. There was slight muscular spasm over the mass; there was no rebound tenderness. On pelvic examination tenderness was elicited high in the right fornix. Rectal examination disclosed slight infiltration of the rectal wall on this side. The remainder of the physical examination revealed no remarkable abnormalities.

The Wassermann and Kahn reactions of the blood were negative. The urea nitrogen was 18 mg. per hundred cubic centimeters. The white blood cell count was 11,000, with polymorphonuclear leukocytes 74 per cent. The hemoglobin was 83 per cent. The sedimentation time was one hour. The blood pressure was 184 systolic and 110 diastolic.

It was felt that this patient was suffering from either an appendical abscess or possibly from a tumor of the cecum, most likely carcinoma.

At operation, April 16, a mass was found within the lumen of the cecum, apparently arising from the ileocecal valve. The nature of this mass could not be definitely determined, and accordingly an exploratory cecotomy was performed. Frozen sections revealed carcinoid tumor. There were numerous lymph nodes in the mesentery, some of which were resected. The ileum itself for the distal 1½ feet (45.5 cm.) was dilated and thickened. The cecotomy opening was closed, the terminal 1½ feet of ileum, appendix and cecum were resected, and an ileotransversocolostomy was performed.

The patient made a good recovery. She had a normal bowel movement on the sixth postoperative day and continued to have normal bowel movements until she was discharged twenty-one days later, markedly improved.

Gross Description of Surgical Specimen (Fig. 1 *A*).—The specimen consisted of a resected portion of the terminal part of the ileum, the cecum and a small portion of the ascending colon. The segment of ileum measured 40 cm. in length. As one approached the ileocecal junction, the wall of the ileum gradually became thickened and more firm and its lumen wider. The serosal surface was pink-gray and somewhat more dulled than normal. The wall of the ileum was thickened to approximately 5 mm. The mucosal surface was markedly congested and showed numerous pinpoint-sized hemorrhages throughout. At the ileocecal junction a firm irregular mass was present. This was produced by a rounded plaquelike tumor, 2.5 cm. in diameter, immediately proximal to the ileocecal valve. Section through the wall at this point revealed the tumor to be composed of firm homogeneous yellowish brown tissue. It could be seen to penetrate all walls of the intestine and to infiltrate the mesentery. The circumference of the ileum (measured from the serosal surface) in the region of the ileocecal valve was 4.5 cm. The ileum directly proximal to this point measured 8 cm. in circumference.

The cecum and ascending colon beyond this showed no gross abnormalities on the mucosal surface. Numerous adhesions were present about the serosal surface.

Several lymph nodes, which were quite firm, were felt in the mesentery. Just external to the mass a packet of nodes was present. These were discrete but adherent to each other. On section they were found to vary in size from 0.5 to 2 cm. in diameter. Opaque homogeneous yellow-brown areas were prominent and formed a striking contrast to the surrounding pale gray lymphoid tissue.

Microscopic Description.—The tumor consisted of groups of cells arranged in compact nests and separated from each other by variable amounts of stroma. The cellular nests varied in size from two to three cells to large sheets of numerous cells. Most of the cell aggregates were of a solid nature, although in some of the smaller ones there was a tendency toward the formation of rosette-like

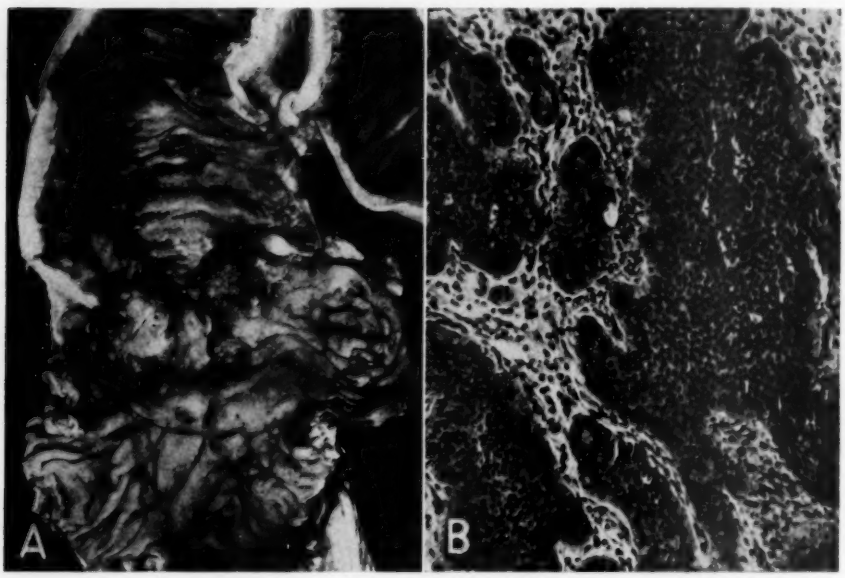

Fig. 1 (case 1).—*A*, specimen showing an annular stenosing argentaffin tumor of the terminal portion of the ileum that had produced intestinal obstruction. *B*, low power photomicrograph of the same tumor, demonstrating the solid cell aggregates and a few pseudoglandular formations.

pseudoglandular structures (fig. 1 *B*). In several large cellular groups there were clear spaces which contained a fine granular eosinophilic débris. No clearly glandular structures were demonstrable. The tendency toward the tubular formation was greater in the vicinity of the mucosa; in the deeper portions pseudoglandular formations were rare. The tumor involved all the intestinal coats.

The neoplastic cells were, for the most part, round or polygonal. The nuclei were spherical or ovoid and situated in the central portions of the cells. They possessed sharply defined membranes and were rich in granular chromatin, uniformly dispersed. Evidence of mitosis was not seen, but a moderate number of amitotic figures were noted. Many nuclei contained large particles of chromatin material resembling nucleoli of basophilic staining reaction. Although usually homogeneous, some cells contained many fine punctate acidophilic granules

Masson's silver stain (fig. 2 *A*) revealed innumerable densely packed brownish black granules, eccentrically situated within the cytoplasm.

Fine spongy vacuolation was a feature of the cytoplasm of some of the cellular aggregates. The vacuolation and granularity of the cytoplasm in many instances obscured the precise definition of the cell boundaries.

Along the periphery of some of the cellular aggregates were seen cylindric cells arranged in a palisade manner, the long axis of the cell perpendicular to the basement membrane. The nuclei were situated in the basal portions of the cells and were similar to those of the polygonal cells, although they showed a more uniform tendency toward an ovoid form.

The cells comprising the rosettes were columnar; their nuclei were situated in the basal portions of the cells and were ovoid. The cytoplasm at the base of the cell was granular and in striking contrast to that in the apical portion, which was homogeneous, pale, faintly eosinophilic and occasionally vacuolated. The cell borders were well defined. The upper portions of the cells formed a sharp circular border which assumed the form of a pseudolumen, in which a homogeneous hyaline eosinophilic globule was usually seen.

The tumor elements were surrounded by a variable amount of connective tissue and smooth muscle. In many instances the myofibrils were distinctly increased in size, frequently to a striking degree. Those portions of the tumor which had infiltrated all coats and the mesentery were more cellular and had produced smaller alveolar clumps. Amitosis was more frequent, and the cells were more pleomorphic; a few multinucleated cells were discernible.

Perineural and perivascular lymphatics in such areas were plugged with tumor cells. Neural invasion could be demonstrated. Several mesenteric lymph nodes revealed peripheral sinuses crowded with tumor cells (fig. 2 *B*). The lymphoid tissue of one node was completely replaced by neoplastic elements. The islands of cells were embedded in a stroma rich in dilated capillaries.

Follow-up.—The patient remained well until July 2, 1935 (two and a half months after operation), when she returned complaining of a sense of a mass at the site of operation. Although there was no biopsy or laparotomy, the nature of the local lesion made it seem probable that the mass was a local recurrence. The patient was accordingly treated with roentgen rays of high voltage from August 16 to September 24, through the following ports: right lower abdominal anterior; right lower abdominal lateral; right lower abdominal posterior. One thousand roentgens were delivered to each port over an area of 10 by 15 cm., the following factors being observed: 200 kilovolts; 50 cm. target skin distance; filtration through 0.5 mm. of copper and 1 mm. of aluminum. The mass decreased in size. On Oct. 9, 1937, two and one half years after operation, the patient was seen again. She had no complaints except of slight diarrhea and had gained 30 pounds (13.5 Kg.) since operation. Physical examination gave negative results. The mass had completely disappeared.

Comment.—This was a case, then, of a malignant argentaffin tumor of the ileum which produced local infiltration, intestinal obstruction, regional metastases and in all likelihood a local recurrence. The observations indicate the possibility of a good prognosis following surgical intervention and suggest that argentaffin tumors may be radiosensitive.

CASE 2.—This case was encountered by Dr. A. A. Berg. A white man 71 years of age had had chronic intestinal obstruction for a number of years. On physical examination, April 30, 1931, there were noted distended intestinal coils, with hyperperistalsis. There was no bowel movement even after enemas, and the patient vomited frequently. The laboratory findings were of no significance.

May 4, 1931, laparotomy revealed an obstructive stricture of the terminal portion of the ileum. The loops of ileum proximal to the point of obstruction were distended; those distal, collapsed. Except for the immediate vicinity of the stricture, the intestinal wall was normal, the stricture apparently being caused by scarring. No enlarged glands were noted in the mesentery. The ileum was resected for a distance of 3 inches (7.5 cm.) on each side of the obstructing portion, and an ileosigmoidostomy was performed. The postoperative course was uneventful and the patient was discharged well, twenty-four days later.

Gross Description of Surgical Specimen.—The specimen consisted of a portion of ileum measuring 18 cm. in length. Proximal to the stenotic portion, it was 10 cm. in diameter. Six cubic centimeters above the distal portion was a broad ulcerating area which involved almost the entire circumference of the ileum. The

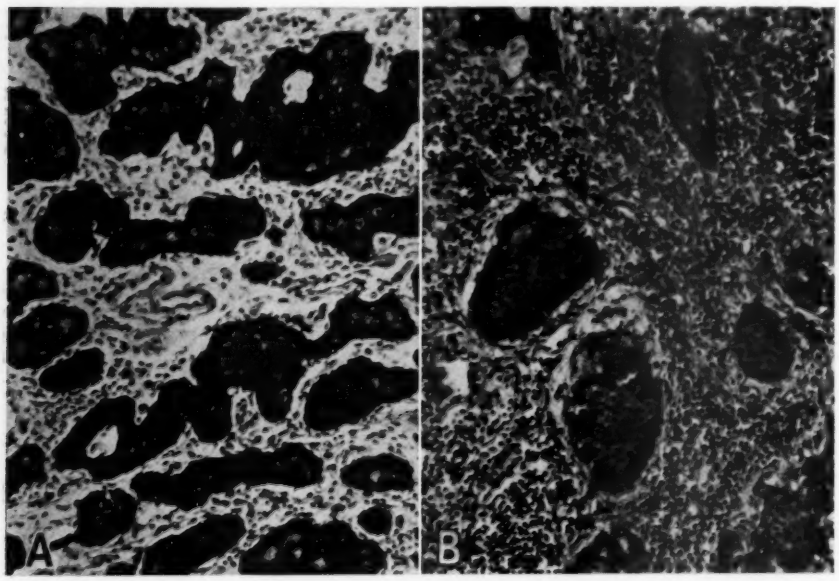

Fig. 2.—*A*, Masson's silver stain of the tumor shown in figure 1, revealing the densely packed argentaffin granules at the bases of the cells. *B*, low power photomicrograph of a metastasis to a lymph node of the tumor shown in figure 1.

wall of this portion of the ileum was markedly thickened and produced a roughened nodular irregularity. Just proximal to this portion was a marked thickening, which measured 3 cm. and was diffusely infiltrated with a lemon yellow firm material, which extended into the mesentery. The mucosa of the ileum away from the tumor mass and the plicae were prominent. Proximal to the involved portion of the intestine, the lumen was distinctly widened and somewhat thickened. The narrowest portion of the specimen measured 5 cm. in diameter.

Microscopic Description.—The tumor cells were arranged in discrete nests, separated by a fibromuscular stroma. The entire wall and mesentery were infiltrated by these cellular nests. In the mesentery the surrounding stroma presented a pronounced fibrohyaline sclerosing reaction. There was a preponderance of

solid alveolar structures, composed of polygonal cells, although pseudoglandular elements were also recognized. The cells were of the same character as those described in the previous specimen.

Follow-up.—Four years after operation, Sept. 24, 1935, the patient was well.

Comment.—This case was one of an obstructive type of argentaffin neoplasm, which extended into the mesentery and responded well to surgical removal.

CASE 3.—This case is included by permission of Drs. J. Ehrlich, M. Cohen and M. Bookman, of Lebanon Hospital, New York.

A 32 year old white man was healthy until one month before admission, when he began to suffer from a griping pain about the umbilicus. This pain lasted about one hour and was accompanied by nausea but no vomiting. The pain was unrelated to meals and was continuous to the time of admission except on two occasions when it had been relieved by sodium bicarbonate. There was no history of constipation. At 3 o'clock in the morning of the day on which he was admitted he began to have severe cramps in the right lower quadrant of the abdomen, followed by vomiting. His appetite had always been good. There had been some loss of weight and strength.

Physical examination, June 22, 1933, revealed a movable tender mass about the size of an egg in the right lower quadrant of the abdomen at McBurney's point. The mass was irregular in outline. It did not move with respirations.

The urine and blood were normal; the Wassermann reaction of the blood was negative.

A preoperative diagnosis was made of intestinal obstruction due to a chronic intussusception, an appendicular abscess or a tumor of the cecum.

At operation, June 24, an ileocolic intussusception was found. This comprised from 10 to 12 inches (25.5 to 30.5 cm.) of small intestine included with the cecum. At a point about 10 inches from the ileocecal junction a tumor was found within the ileum, which was attached near the mesenteric border of the bowel by a broad base. The mass was the size of a walnut. At the serosal side of the intestine there seemed to be a dense fibrous scar. The intussusception was delivered and reduced, and the segment of intestine containing the tumor was resected. The postoperative course was uneventful.

Gross Description of Surgical Specimen (Fig. 3 *A*).—The specimen consisted of a resected portion of small intestine which measured 7.5 cm. in length and 4.5 cm. in circumference. Situated on one aspect, at the junction of the middle and the lower third of the specimen, was an oval mass which projected into the lumen and measured 4 by 1.5 cm. The entire thickness of the wall at this point was diffusely increased, and yet each intestinal layer was discernible with ease. The mucosa was thin, the submucosa was most markedly involved in the thickening and was lemon yellow, and the serosa was markedly thickened and similarly colored. The projecting mass assumed a sessile polypoid character. The mucosa of the intestine beyond this polypoid projection presented no remarkable gross changes. The serosa over the tumor mass was dimpled but otherwise was smooth and glistening.

Microscopic Description (Fig. 3 *B*).—The tumor was characterized by the presence of numerous small polygonal cells arranged in compact masses, most of which were solid structures, but some tended to assume pseudoglandular forms. The cells were uniform in size and contained round nuclei, in which were moderate amounts of coarse chromatin granules. No mitosis was noted. The cytoplasm was pale and almost uniformly homogeneous and not infrequently contained

eosinophilic granules. Vacuolation was not prominent. The stroma consisted of dense muscular and fibrous connective tissue. The tumor infiltrated all coats of the intestine.

Follow-up.—The patient Sept. 7, 1935, two years after operation, was found in good health.

Comment.—This case was one of a small sessile carcinoid tumor of the terminal portion of the ileum producing an ileocolic intussusception.

CASE 4.—A 50 year old man had been in the hospital on numerous occasions for chronic bronchiectasis and bronchiolitis and had been receiving treatment with roentgen rays of high voltage in an attempt to control hemoptysis. Physical examination revealed a man chronically ill and coughing moderately. The chest

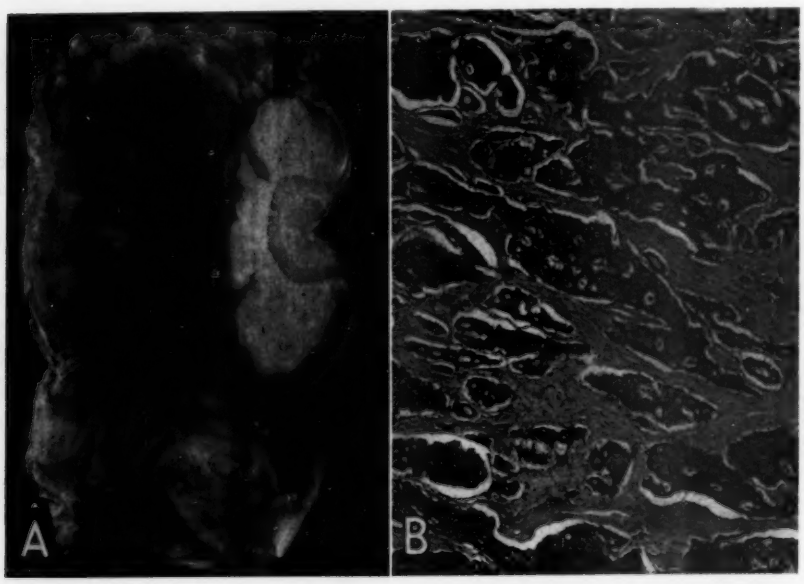

Fig. 3 (case 3).—*A*, specimen showing a carcinoid of the terminal portion of the ileum that had produced an intussusception. *B*, photomicrograph of the same tumor.

was emphysematous and the breath sounds hyperresonant. Scattered rales could be heard throughout the lower lobe of the right lung; the base moved poorly. The examination otherwise gave negative results. The patient did not respond to therapy and died eight days after admission.

Necropsy.—The observations were: diffuse cylindric and saccular bronchiectasis with acute and chronic bronchitis, bronchiolitis and pneumonitis; fibrosis and pleural adhesions of all lobes; healed tuberculosis, with primary infection of the upper lobe of the left lung; hypertrophy of the right ventricle of the heart; dilatation of the left ventricle (slight); acute infectious splenic swelling; fatty changes of the liver; a carcinoid of the terminal portion of the ileum with a metastasis in the liver; a hemangioma in the medulla of the right kidney; a thrombosed varix of the esophagus; a patent foramen ovale.

The cause of death was bronchiectasis with acute and chronic pneumonitis.

Gross Description of Carcinoid (Fig. 4*A*).—Within the terminal portion of the ileum, 10 cm. proximal to the ileocecal valve, was a cherry-sized firm round submucosal node, 1.5 cm. in diameter, the sectioned surface of which was smooth and homogeneously yellow. The tumor mass beneath the thin mucosa was freely movable. The serosa formed the external boundary of the mass and was smooth and gray. The mucosa of the ileum surrounding the mass presented no remarkable changes. There was found on the anterior surface of the right lobe of the liver a pinhead-sized slightly raised, well defined soft yellow nodule.

Microscopic Description of Carcinoid.—Sections made through the tumor revealed nests of polygonal cells separated by a variable amount of fibrous stroma. The cells assumed an alveolar arrangement. They were uniform for the greater part and contained moderate-sized vesicular nuclei with discrete chromatin

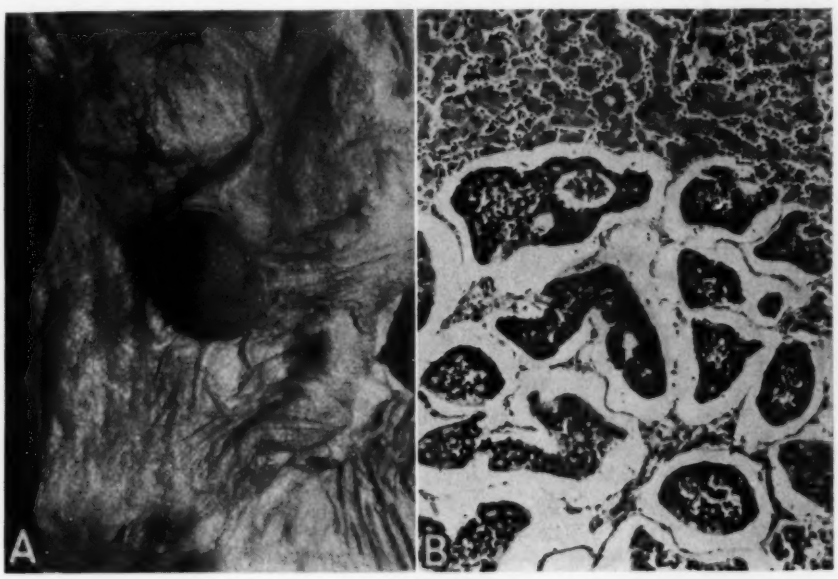

Fig. 4 (case 4).—*A*, specimen showing a small sessile polypoid carcinoid of the ileum which had produced a metastasis to the liver. *B*, low power photomicrograph of the metastatic nodule in the liver produced from the carcinoid in *A*.

granules. Occasionally small polygonal cells with pyknotic nuclei were found, and intermediary forms between these and the more common type were recognized. The cytoplasm was pale, granular and vacuolated. At the periphery of the tumor the trabeculations of the stroma were coarser and predominantly of a thick fibromuscular character. This was noted particularly in the muscularis, which was infiltrated with tumor cells. Neoplastic cells were seen within several lymphatics. Dilated capillaries were prominent at the periphery.

The tumor nodule (fig. 4*B*) in the liver consisted of groups of cells compactly arranged in solid nests. The cells were identical with the primary tumor of the ileum. A thin, delicate connective tissue stroma separated the cellular

aggregates. A few remnants of liver cells were recognized in some of the larger trabeculae. The lesion was sharply demarcated from the surrounding liver tissue but not encapsulated.

Comment.—This was a case of a carcinoid tumor of the terminal portion of the ileum metastasizing to the liver.

CASE 5.—A 51 year old man was admitted to the medical service of the Mount Sinai Hospital with a long history of gastric intestinal complaints. Since the age of 15 he had had bouts of watery diarrhea which lasted from two to three days and recurred at monthly intervals. From the age of 35 to that of 40 each spell terminated with a flow of bright red blood. Five years previous to admission he experienced an episode of severe pain in the right upper quadrant of the abdomen of three days' duration. No other symptoms were associated with the pain. For four years previous to admission, more particularly during the last five months, there had been almost constant watery stools. He had also experienced during the latter period severe pain in the right upper quadrant of the abdomen. There had been a loss of 10 pounds (4.5 Kg.) in weight during the year previous to admission.

On physical examination a rather distinct mass was palpable in the right side of the abdomen.

The blood pressure was 148 systolic and 94 diastolic. The white blood cell count was 8,300; the polymorphonuclear leukocytes were 64 per cent; the hemoglobin, 85 per cent. The stools were persistently positive for blood when tested with guaiac. The Wassermann reaction of the blood was negative.

Roentgenologic examination disclosed narrowing of the duodenal bulb, an irregular zone of constriction at the hepatic flexure and narrowing of the terminal half inch of the ileum. The ascending colon was also irregularly filled.

A laparotomy, March 18, 1933, revealed the presence of a thickened, firm lesion in the ascending colon just proximal to the hepatic flexure. An ileotransversocolostomy was performed, with resection of the intervening portion of intestine. The patient did very poorly, however, and died soon after the operation.

Necropsy.—The observations were: status after a colonic resection with side to side ileotransversostomy for polyposis of the hepatic flexure with carcinomatous changes; hemoperitoneum; polyposis of the transverse and descending colon without carcinomatous changes; congestion of the ileum and of the liver; metastases of carcinoma in the regional lymph nodes.

Gross Description of Surgical Specimen.—The specimen consisted of the cecum and a portion of the ascending colon, together measuring approximately 28 cm. in length, and 7 cm. of the terminal portion of the ileum. The appendix was also attached and measured 5 cm. in length. In the intestinal wall at the terminal portion of the ileum, immediately adjacent to the ileocecal valve, was a globoid firm nodule, 1 cm. in diameter and covered by intact mucosa. The nodule was situated within the mucosa and submucosa and sharply separated from the muscularis. The sectioned surface of the mass was smooth, glistening, opaque and light yellow. The remainder of the ileum showed no significant changes. Its circumference at the site of the tumor was 5 cm. and was equal to that of the remainder of the resected portion of the ileum. The ascending colon was 15 cm. in circumference at its cecal portion but narrowed down to 11 cm. at its distal extremity. Arising from the mucosa of the colon were numerous polypoid projections, varying from 5 mm. to 3 cm. in diameter. Nine centimeters from the ileocecal valve the mucosa presented elongated and numerically increased

annular folds, which in some instances were 1 cm. in height. From these there projected numerous polypoid excrescences. In addition to the numerous polypoid structures projecting from the mucosal folds in this region there were other polyps scattered over the surface of the cecum and ascending colon. Some of these were sessile and possessed broad bases. One measured 1.5 cm. in diameter and was of firmer consistency. A section through it revealed opaque whitish tissue which infiltrated the submucosa and muscularis. Adjacent to this firm plaquelike area, another portion of mucosa, approximately 2 cm. in diameter, was thickened and infiltrated. The cut section revealed similar changes. The underlying fat was abundant and contained large lymph nodes, which on section presented mottled gray surfaces.

Microscopic Description.—The polypoid growths of the colon were composed of large branching glands, irregular in shape and size. The cells were cylindric and lined well defined lumens. Their nuclei were uniformly ovoid, contained moderate amounts of chromatin and were situated in the basal portions of the cells. The cellular cytoplasm was pale and contained vacuoles in the form of large flasklike hollow areas occupying the apical portions of the cells. Groups of glands were often arranged on thin connective tissue stalks containing small blood vessels.

In carcinomatous areas the orderly arrangement was lacking. Structures of irregular shape and size and composed of cells varying in form from low cuboidal to high columnar and displaying marked pleomorphism were seen. They contained round to ovoid nuclei, rich in chromatin and containing one or two eosinophilic nucleoli. Occasional mitotic figures were noted. The cells had lost their polarity, lined the irregular glands in several layers and in many instances had penetrated their basement membranes and infiltrated the intestinal wall and mesentery. The characteristic structure was an irregular but recognizable counterpart of the intestinal glands. The stroma was composed of fibrous connective tissue, rich in lymphocytes, plasma cells and leukocytes.

An omental lymph node was almost completely replaced by distorted glandular elements which combined all the cytologic and morphologic features of the primary infiltrating carcinoma of the bowel.

The yellow tumor noted in the ileum possessed entirely different characteristics. It consisted of cells the majority of which were polygonal and arranged in alveolar masses. There was a tendency toward the formation of tubular pseudoglandular structures; many of these contained homogeneous colloid-like material. The nuclei were small and hyperchromatic and contained abundant fine and coarse chromatin material. No nucleoli were noted. Numerous amitotic figures were discerned but no true mitosis. The cytoplasm was of moderate amount and granular and contained numerous small vacuoles. Masson's silver stain revealed numerous black granules at the bases of most of the cells. A fibromuscular stroma was abundant and divided the tumor into many discrete nests. With the exception of one small isolated cell nest, which was found occupying the space between two muscle bundles, the tumor was well defined and confined to the mucosa, submucosa and innermost layer of the muscularis.

Comment.—This was an unusual instance of the simultaneous occurrence of benign adenomatous polypi, an adenocarcinoma of the ascending colon and a carcinoid of the terminal portion of the ileum in the same surgical specimen. Several regional lymph nodes disclosed carcinomatous infiltration.

Case 6.—A 56 year old white man had been well until six years before admission, when he became dyspneic on exertion and experienced pain in the right scapular region. He had a history also of occasional constipation alternating with

slight diarrhea. Recently this complaint had become more bothersome. Physical examination, April 30, 1930, revealed an acutely ill, cyanotic, dyspneic man. The breath sounds were diminished. The heart was enlarged, and the heart sounds poor; the blood pressure was 98 systolic and 70 diastolic. The urine showed albumin (4 plus). The patient's course was steadily downhill, and death occurred seven days after admission.

Necropsy.—The observations were: coronary sclerosis with thrombosis of the left anterior descending artery; aneurysm of the left ventricle with a mural thrombus; hypertrophy and dilatation of the right and left ventricles; multiple infarcts of the right kidney, spleen and right lung; an adenoma of the left adrenal; an adenoma of the prostate; a carcinoid tumor of the ileum; arteriosclerosis and arteriolosclerosis of the kidneys.

Gross Description of Carcinoid.—There was a superficially ulcerated area about 1.5 cm. in diameter in the lower part of the ileum.

Microscopic Description.—The tumor mass consisted of islands of cells separated from each other by a dense fibromuscular stroma. Most of the islands were solid, though in several rosette formations could be discerned. It extended through the mucosa, submucosa and circular muscle layer to the layer of longitudinal muscle, where it was sharply circumscribed. Tumor elements were situated in the lymphatics outside the main bulk of the nodule. They were similar in all respects to those forming the main tumor and were characterized by large spherical nuclei of a more vesicular nature than had been observed in the most typical lesion. They formed a striking contrast with cells which contained small pyknotic irregular nuclei and were scattered in larger and smaller islands in the large submucosal nodule. These occurred in the same alveolar clumps as the large relatively pale cells. Intermediary forms were noted in which the coarsely granular pyknotic nuclei showed dispersion and clumping of chromatin granules with the formation of fewer but larger karyosomes. The cytoplasm of these forms was fine and more abundant and stained faintly with eosin. Many cells of this type had ovoid nuclei. There were other forms in which sharp definition of the nuclear membrane became prominent, and further isolation and enlargement of the chromatin material became evident. These already closely resembled the cells characterized by large vesicular nuclei and scattered coarse chromatin granules, which formed a prominent feature in the cytologic picture of this tumor.

The Bielschowsky stain revealed an intricate ramifying series of interconnected argyrophilic fibrils which surrounded the tumor elements and separated the alveolar clumps. No definite relationship between the cellular and fibrillar elements was evident.

In the area adjacent to the tumor the stroma was very vascular and contained numerous middle-sized arterioles, engorged with blood. The stroma of the tumor contained many fine capillaries.

CASE 7.—A 79 year old man was admitted to the hospital Nov. 6, 1927, because of shortness of breath and swelling of the feet of two months' duration. He had been admitted on previous occasions with a diagnosis of retroperitoneal lymphosarcoma and had received roentgen therapy. At the present admission a diagnosis of emphysema, chronic bronchitis, essential hypertension, myocardial insufficiency, generalized arteriosclerosis and arteriosclerotic heart disease was made. The patient responded poorly and died Nov. 29, 1927.

Necropsy.—At autopsy there were found from ten to fifteen small pea-sized nodules scattered throughout the jejunum (fig. 5 *A*). These were very firm and

apparently extended down as far as the muscularis. They were well circumscribed, and each projected from a broad base into the lumen.

Microscopic Description.—Microscopically, these nodules were composed of tumor cells which were situated between the submucosa and the internal muscular coat of the intestine. The cells were arranged in alveolar masses and were composed of round regular nuclei and pale eosinophilic granular cytoplasm.

A neighboring lymph node revealed the presence of typical carcinoid cells arranged in nests, which were separated by a moderate amount of fibrous connective tissue.

Comment.—This case was one of multiple carcinoid tumors of the jejunum metastasizing to a lymph node.

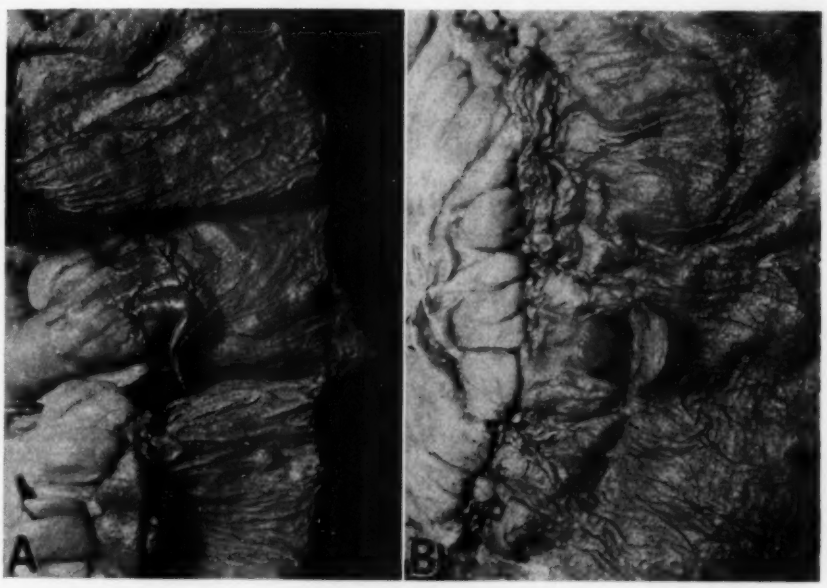

Fig. 5.—*A* (case 7), specimen demonstrating multiple small polypoid carcinoids of the jejunum, which had produced metastasis to lymph nodes. *B* (case 8), photograph of a small polypoid benign carcinoid of the terminal portion of the ileum.

CASE 8.—A white man died of myocardial failure a few hours after admission to the hospital, Dec. 14, 1927, and no clinical data were obtained.

Gross Description of Carcinoid.—Autopsy revealed a small pea-sized firm nodule in the terminal portion of the ileum, which protruded into the lumen but was completely covered by, and attached to, the mucous membrane (fig. 5 *B*). It apparently projected down as far as the serosa, on which surface there was a slight irregular elevation but no peritoneal reaction.

Microscopic Description.—Microscopically, this nodule proved to be a typical carcinoid tumor. Lymph nodes in the vicinity of the tumor revealed no evidence of neoplastic tissue.

CASE 9.—This case is reported by permission of Drs. B. Crohn and A. A. Berg. A white man 54 years old had been under the care of various physicians for twenty years for diarrhea and "intestinal disturbances." His complaints were believed to be on a functional basis.

On admission to the hospital, July 7, 1937, he complained, in addition, of pain in the right iliac fossa. Physical examination revealed a white man in good general condition. There was no enlargement of the liver. On rectal examination, marked induration and swelling in the right iliac fossa and right side of the pelvis were noted. Lymphadenopathy was not observed.

The hemoglobin was 96 per cent; the red blood cell count, 4,990,000; the white blood cell count,, 6,000; the polymorphonuclear leukocytes were 74 per cent; the lymphocytes, 25 per cent; the monocytes, 4 per cent. The urine revealed no abnormalities.

The patient had had numerous roentgen examinations of the gastrointestinal tract during the past twenty years, all of which were reported to have shown nothing abnormal. Prior to the present admission to the hospital, a roentgen examination revealed a filling defect in the caput coli and an irregularity of the terminal portion of the ileum. The findings were similar to those produced by regional ileitis with involvement of the caput coli.

The patient was accordingly operated on. The peritoneum was free from fluid. The terminal 8 inches (20 cm.) of the ileum was found to be markedly dilated and the wall somewhat thickened. An area of constriction was found approximately 3 inches (7.5 cm.) from the ileocecal valve. The latter was markedly thickened and its lumen narrowed. The mesentery of the terminal portion of the ileum was thickened, and enlarged lymph nodes were observed in the mesentery near the terminal portion of the ileum.

The terminal part of the ileum, cecum, ascending colon and proximal half of the transverse colon were resected and a side to side ileotransversocolostomy performed.

The patient made an uneventful recovery and was discharged in good condition Aug. 19, 1937 (forty-three days after operation and about twenty-one years after the onset of symptoms).

Gross Description of Surgical Specimen (Fig. 6).—The specimen consisted of about 40 cm. of ileum, the cecum and about 15 cm. of ascending and transverse colon. Attached to the ileum was a segment of mesentery, 7 cm. at its widest point, and within this mesentery were found numerous lymph nodes. At a distance of 5.5 cm. from the point of proximal resection there was an almost round plaque, 12 by 10 mm. in diameter and raised 3 mm. above the surface; it was firmly adherent to all the layers of the intestine but not ulcerated. The folds of the intestines, however, ended sharply at the tumor. On the serosal aspect the tumor appeared smaller and slightly umbilicated. In the further course of the intestine the mucosa appeared perfectly normal, but the wall gradually became thickened owing to hypertrophy of the muscle layers. The mucosa and submucosa gradually became edematous. Nine centimeters proximal to the ileocecal valve was another tumor, situated at the mesenteric border and bulging 3 mm. over the mucosa. This was sessile and elongated, measuring 27 by 17 mm. in diameter; it was not ulcerated but infiltrated the entire mucosa and submucosa and appeared also on the serosal surface. By its projection into the lumen and its infiltrative growth, it produced marked narrowing. Opposite this tumor, several smaller yellow nodules the size of split peas were found situated within the mucosa. The subsequent portion of the intestine was again slightly dilated,

measuring 10 cm. in circumference. Just at the ileocecal junction, but still within the ileum, there was still a third tumor, 30 by 18 mm. in diameter and 12 mm. high, which infiltrated throughout the mucosa and submucosa. The serosa over this tumor was thickened, and the fat tissue at the ileocecal angle was adherent to it. This tumor was surrounded by at least seven smaller tumors as satellites, each about the size of a pea. The width of the ileocecal valve was taken up by a ridgelike tumor, which almost encircled the lumen and was raised about 13 mm. above the mucosa. It also was surrounded by numerous smaller tumors as satellites, and several smaller tumors were still found within the mucosa of the

Fig. 6 (case 9).—Multiple argentaffin tumors of the terminal portion of the ileum, ileocecal valve and ascending colon which had produced intestinal obstruction and widespread metastases to regional lymph nodes.

beginning of the ascending colon. The further course of the ascending colon showed a normal smooth mucosa; within the ileocecal angle there were numerous nodes, which were matted together to form, with the adjacent fat tissue, a mass which measured 40 by 20 mm. in diameter.

The appendix was opened. The mucosa showed no abnormalities. However, the entire appendix was adherent to the tumor mass. A portion of the adjacent omentum, measuring 8 by 10 cm., appeared soft throughout; no nodules and no masses were noted.

TABLE 1.—*Review of Cases of Small Intestinal Carcinoids Reported Since 1930*

Author	Age	Sex	Location of Tumor	Symptoms	Location of Metastases	Comment
Busser.....	38	F	Ileum	Obstruction revealed by roentgenogram	None	Death due to bronchopneumonia
Gáspár.....	74	F	Jejunum	Obstruction revealed by roentgenogram	Mesentery and liver	Death from carcinoid of small intestine with obstruction
Cope and Newcomb	42	M	Ileum	Obstruction	Peritoneum (pelvic masses), testis	Metastases resected; patient well
Newcomb.....	?	?	Ileum	?	Lymph glands	?
Stewart, cited by Cope and Newcomb	?	?	Ileocecal	?	Lymph glands	?
	?	?	Small intestine	?	Lymph glands, peritoneum, liver	?
Pack and Davis..	73	M	Lower part of ileum	Slight stenosis	Mesenteric lymph nodes	Death from cardiac-vascular-renal disease
Planson.....	41	M	Terminal portion of ileum	Complete obstruction	Inguinal, crural and mesenteric lymph nodes	Resection; patient well
Ritchie.....	51	F	Middle portion of intestine	None	Liver and mesenteric lymph nodes	Death due to peritonitis subsequent to resection
	71	F	Ileum	Obstruction	None	Death following iliocostomy
	60	F	?	Obstruction	None	Resection; results not mentioned
	?	?	?	Obstruction	None	Resection; results not mentioned
Carr.....	61	M	Ileum	Obstruction revealed by roentgenogram	None	?
	51	F	Ileum	Obstruction revealed by roentgenogram	Mesentery and mesenteric lymph nodes	Resection; patient well
Marangos.....	68	F	Middle of ileum	?	Mesenteric lymph nodes and liver	Results not mentioned
	68	F	Terminal portion of ileum	?	Mesenteric lymph nodes	Results not mentioned
	59	M	Ileum	None	?	Results not mentioned
	67	F	Terminal portion of ileum	None	?	Results not mentioned
Kaufmann.....	63	M	Ileum	None	None	?
Deml.....	34	M	Ileum	None	None	Death from angina and sepsis
Ewell and Jackson	59	F	Terminal portion of ileum	Ogstruction revealed by roentgenogram	None	Resection; patient well
Glesne.....	52	F	Lower part of ileum	Obstruction	None	Death from pneumonia after operation
Pallos.....	65	M	Lower part of ileum	Obstruction	None	Death from pneumonia after operation
Scholte.....	46	M	Ileum	None	Mesentery, mesenteric nodes and liver	Death due to cardiac failure

TABLE 1.—*Review of Cases of Small Intestinal Carcinoids Reported Since 1930—Continued*

Author	Age	Sex	Location of Tumor	Symptoms	Location of Metastases	Comment
Möri.....	26	M	Ileum	Obstruction revealed by roentgenogram	Mesenteric lymph nodes	Resection; death due to pneumonia
	51	M	Ileum	None	Lymph nodes	Operation for carcinoma of stomach; death from peritonitis
Govaerts.....	64	F	Ileum	Obstruction revealed by roentgenogram	None	Death after operation for carcinoma
Meeker.....	61	?	Ileum	Obstruction	Mesenteric lymph nodes	Resection; results not recorded
Stout.....	55	M	Ileum	Obstruction	Mesenteric lymph nodes	Death after resection
Raiford.....	49	M	Duodenum	Obstruction	None	Excision; patient well
	45	F	Terminal portion of ileum	Obstruction	Lymph nodes and peritoneum	Death due to metastases
	57	M	Ileum	None	Regional lymph nodes and liver	Death due to bronchopneumonia
	44	F	Ileum	None	None	Death due to peritonitis after operation for appendicitis
	62	M	Ileum	None	None	Death due to pneumonia
	50	M	Jejunum	None	None	Death after operation for carcinoma of stomach
	66	F	Ileum	None	None	Death due to ruptured aneurysm
	60	M	Ileum	None	None	Death from myocardial failure
	55	M	Ileum	None	None	Death due to pneumonia
Cabot case 20342	64	F	Ileum	Obstruction revealed by roentgenogram	None	Resection; patient well
Christopher.....	55	F	Ileum	Obstruction	None	Resection; patient well
Lee and Taylor..	51	F	Ileum	Obstruction	Mesenteric lymph nodes	Resection; patient well
	53	F	Ileum	Obstruction	Subserous fat and regional lymph nodes	Resection; patient well
Humphreys.....	60	M	Ileum	Obstruction	Mesenteric lymph nodes	Death due to pneumonia
	66	M	Ileum	None	Regional lymph nodes	Death due to carcinoma of esophagus
	47	M	Ileum	Obstruction	Regional lymph nodes	Death due to cardiac failure
	?	?	Jejunum	None	None	?
	?	?	Ileum	None	None	?
	?	?	Ileum	None	None	?
	?	?	Ileum	None	None	?
Warren and Gates	53	F	Ileum	Obstruction	None	Death with evidence of intestinal obstruction
Price.....	54	F	Meckel's diverticulum	Obstruction	None	Death from paralytic ileus after operation

TABLE 1.—*Review of Cases of Small Intestinal Carcinoids Reported Since 1930—Continued*

Author	Age	Sex	Location of Tumor	Symptoms	Location of Metastases	Comment
Hertzog and Carlson	54	M	Meckel's diverticulum	None	None	?
	58	M	Meckel's diverticulum	None	None	?
Lewis and Geschickter	47	M	Ileum	Obstruction	Mesenteric lymph nodes	?
Feyrter* (1934)...	58	M	Duodenum	None	None	?
	40	M	Doudenum and pyloris	None	None	?
	58	M	Duodenum	None	None	?
	88	M	Duodenum	None	None	?
	71	M	Ileum	None	Tumor growing into vein	?
	60	M	Ileum	None	None	?
	72	M	Ileum	Obstruction	Mesenteric lymph nodes; liver	Death from peritonitis following resection
	52	F	Ileum	Obstruction	Mesenteric tissue	Death from peritonitis following resection
	72	F	Jejunum	None	Liver	?
	?	?	Papilla of Vater	None	None	?
Bailey.....	59	M	Jejunum	None	None	Death from carcinoma of stomach
	66	M	Ileum	None	None	?
	71	F	Ileum	None	None	Death from myocardial infarct
	41	F	Ileum	None	None	Death from rheumatic heart failure
	64	F	Ileum	None	None	?
	36	M	Ileum	None	None	Death from tuberculoma of brain
	57	M	Ileum	None	Mesentery	?
	64	M	Ileum	Obstruction revealed by roentgenogram	Mesentery, liver	Tumor resected, metastases left in; patient well 6 months after operation
Gierlich.....	71	M	Lower portion of ileum	None	Perigastric, peripancreatic, mesenteric and retroperitoneal lymph nodes	Death from carcinoma of stomach
	42	M	Ileum	None	None	?
	72	M	Middle portion of small intestine	None	None	?
Knauer.....	52	M	Terminal portion of ileum	Pain in lower part of back; unable to walk	Mesenteric lymph node, liver, dura and compression of spinal cord	Death from carcinoid and its metastases
Wood.....	62	F	Ileum	Obstruction	Regional lymph nodes	Resected; patient well
Jones.....	52	M	Ileum	Obstruction with intussusception	None	Patient well
Merke.....	63	M	Terminal portion of ileum	Acute obstruction	Regional lymph nodes	Tumor not resected but anastomosis performed; patient died from pneumonia 5 years later; no symptoms from tumor

* Feyrter mentioned 32 additional cases.

Microscopic Description of Carcinoids.—The various tumors of the ileum, ileocecal valve, ascending colon and lymph nodes consisted of the same cellular elements. Solid nests of polygonal cells, varying from a few cells to larger aggregates, separated by a fibromuscular stroma, were noted. There was a scarcity of rosette formations. The cellular outlines were indistinct, and the cytoplasm was of a pale eosinophilic color. The spherical nuclei contained discrete chromatin granules. The nuclear membranes were distinct. Neoplastic cells were noted within several lymphatic channels. Masson's silver stain revealed the presence of numerous densely packed argentaffin granules within the cytoplasm of the tumor cells. All coats of the intestinal wall demonstrated neoplastic cells.

Regional lymph nodes revealed the presence of neoplastic cells identical in appearance with those of the intestine.

Follow-Up.—An observation six months after operation revealed marked clinical improvement. At the present time the patient is receiving treatment with roentgen rays of high voltage in view of the widespread involvement of the regional lymph nodes.

Comment.—This case demonstrated multiple carcinoids of the terminal portion of the ileum, ileocecal valve and ascending colon with extensive metastases in lymph nodes and intestinal obstruction. The roentgen findings were interesting so far as they resembled the findings in cases of terminal ileitis with involvement of the cecum.

The patient responded well to surgical resection. In view of the widespread involvement of the regional lymph nodes, he is at the present time receiving treatment with roentgen rays of high voltage.

CASE 10.—This was the fourth admission to the Mount Sinai Hospital of a 42 year old Jewish housewife. A clinical diagnosis was made of bronchiectasis of the left lung, rheumatic heart disease with auricular fibrillation and congestive heart failure, chronic cholecystitis and cholelithiasis. A phrenicectomy was performed for pain in the chest, believed due to pleural adhesions. The patient died twelve hours after the operative procedure.

Necropsy.—The observations were: rheumatic heart disease with healing verrucous endocarditis of the aortic valve; insufficiency and stenosis of the aortic valve; insufficiency of the mitral valve; interstitial valvulitis of the tricuspid valve; hypertrophy and dilatation of all chambers of the heart; chronic adhesive pericarditis; bronchiectasis and extensive fibrosis of the left lung; marked shift of the mediastinum to the left; emphysema of the right lung; brown induration of the lungs; moderate arteriosclerosis of the pulmonary arteries; congestion of the spleen, liver, intestines and kidneys; severe fatty changes of the liver; phlebosclerosis of the inferior vena cava; cholelithiasis and choledocholithiasis; small submucous myofibroma of the esophagus; carcinoid of the terminal portion of the ileum.

Gross Description of Carcinoid.—In the lower part of the ileum was a small submucous nodule which measured 8 mm. in diameter. The overlying mucosa was intact.

Microscopic Description of Carcinoid.—The nodule was composed of tumor cells situated between the mucosa and the submucosa. The cells were arranged in alveolar masses and were composed of spherical nuclei and pale eosinophilic cytoplasm. Masson's silver stain revealed numerous densely packed argentaffin granules within the cytoplasm. The morphology was that of a typical carcinoid tumor.

CASE 11.—A 62 year old man was admitted to the Mount Sinai Hospital, April 24, 1937, with a five year history of postprandial pain which was relieved by alkalis. There was a history of several episodes of tarry stools. Three days before admission he was seized with severe paraumbilic crampy pains, and eighteen hours after the onset, on several occasions, he brought up frank blood and "coffee ground material." Physical examination revealed an acutely ill, well nourished, white man; the heart and lungs were normal; there were marked deep and rebound tenderness in both lower quadrants of the abdomen. The hemoglobin was 84 per cent; the blood pressure was 160 systolic and 90 diastolic; the urinalysis showed no abnormality.

The patient was given a continuous intravenous injection of dextrose, and a laparotomy was done. Free fluid was found in the peritoneal cavity, and a ruptured duodenal ulcer was noted. The ulcer was sutured and an appendectomy done. The postoperative course was stormy. The temperature rose rapidly to 105 F., and the patient died on the third postoperative day.

Necropsy.—The main observations were: status of the second day after operation for perforated chronic peptic duodenal ulcer and appendectomy; acute diffuse fibrinopurulent peritonitis; bronchopneumonia of the lower lobes of the right and left lungs; dilatation of the right auricle and ventricle; hypertrophy of the left ventricle; acute esophagitis; a carcinoid of the ileum; cystitis cystica; fibroadenomatous hyperplasia of the prostate; prostatic calculi; polyp of the ascending colon.

The cause of death was the acute diffuse fibropurulent peritonitis.

Gross Description of Carcinoid.—In the lower portion of the ileum, approximately 4½ feet (137 cm.) from the ileocecal junction, the mucosa was raised by a round firm nodule, 8 mm. in diameter. This nodule was fixed to the mucosa and sharply demarcated from the submucosa, into which it protruded. On cut section, the surface was homogeneously yellow. The serosa over the nodule showed no changes.

Microscopic Description of Carcinoid.—The tumor was characterized by the presence of numerous polygonal cells arranged in compact masses, separated by a fibromusclar stroma, typical of a carcinoid tumor.

COMMENT

Incidence.—It is difficult to determine the actual frequency of carcinoids of the small intestine because their occurrence in locations outside the appendix is not generally recognized, and, as Rankin and Mayo pointed out, they are frequently confused with adenocarcinoma.

In 1930 Cooke presented 11 cases of small intestinal carcinoids and collected 104 additional instances from the literature. Since 1930 I have found 111 instances in the literature of small intestinal carcinoids, which number, with the 11 reported here, brings the total to 237.

Nevertheless, the actual frequency is not great. Raiford recorded 29 carcinoid tumors (9 of the ileum) among 62,000 specimens (both autopsy and surgical material) at the Johns Hopkins Hospital. There were 1,611 tumors of the gastrointestinal tract, placing the incidence of carcinoids at 0.18 per cent.

Humphreys encountered 8 cases of carcinoids of the small intestine in a series of 3,200 autopsies, an incidence of 0.22 per cent.

In the series of 47,045 specimens (5,745 autopsy and 41,300 surgical specimens) of the Mount Sinai Hospital there were 31 carcinoids, an incidence of 0.065 per cent of all the material available for study. Of these, 21 involved the appendix (0.044 per cent) and 10 the small intestine (0.021 per cent).

It may be of interest to evaluate the relative incidence of carcinoids as a factor in the general pathologic involvement of the small intestine (table 2). Among 41,300 surgical specimens there were 278 resected portions of small intestine. Of 48 resected neoplastic growths of the small intestine, 4 were carcinoids. Neoplasms constituted 17.2 per cent of all resected small intestinal lesions. Carcinoid tumors composed

TABLE 2.—*Incidence of Carcinoid Tumors at Mount Sinai Hospital in the Period from 1925 to 1937*

	Surgical Material	Autopsy Material	Total
Specimens	41,300	5,745	47,045
Lesions involving small intestine	278	571	846
Neoplasms of small intestine	48	72	120
Carcinoids of:			
(a) Ileum and jejunum	4	6	10
(b) Appendix	19	2	21
Percentage of small intestinal carcinoids among lesions of small intestine	1.4%	1.0%	1.1%
Percentage of small intestinal neoplasms among lesions of small intestine	17.2%	12.6%	14.1%
Percentage of small intestinal carcinoids among neoplasms of small intestine	8.3%	8.3%	8.3%
Total incidence of all carcinoids (appendical and intestinal)	0.059%	0.13%	0.065%

8.3 per cent of all resected small intestinal neoplasms and 1.4 per cent of all lesions of the small intestine which demanded operative intervention.

In the necropsy series there were 72 neoplasms of the small intestine. Of these 6 were carcinoids. The incidence of neoplasms is 12.6 per cent of all involvements of the small intestine, and carcinoid tumors constitute 8.3 per cent of the small neoplastic growths and 1 per cent of all lesions of the small intestine.

Thus carcinoids constitute 8.3 per cent of all observed neoplasms of the small intestine and 1.1 per cent of all involvements of this region by disease processes.

Among 2,373 neoplasms of the entire gastrointestinal tract observed in this series the incidence of carcinoids is 1.3 per cent, and carcinoids of the small intestine represent 0.42 per cent.

Location.—Kultschitzky, or argentaffin, cells are found throughout the entire gastrointestinal tract but are most abundant in the appendix and terminal portion of the ileum. They are usually situated at the bottom of the crypts of Lieberkühn. It is theoretically possible for

carcinoids to develop anywhere in the intestinal glandular mucosa. Carcinoids occur most frequently, however, in the region of the ileocecal valve: 96.2 per cent of the group reported here and 85 per cent of Raiford's series. Although they are most frequently found in the appendix, many occur in the ileum. They have occasionally been reported in the colon, jejunum, duodenum, Meckel's diverticulum and the stomach.

Sex and Age.—There is no definite relation of incidence to sex. Cooke, reviewing 104 cases, states that 50 occurred in males and 43 in females. In the 69 cases in which the sex of the patient was recorded since Cooke's report, 41 occurred in males and 28 in females. In the present group 9 occurred in males and 2 in females.

The average age of patients with small intestinal carcinoids whose cases have been recorded in the literature since 1930 was 57½ years. In the group reported here it was 56⅓ years. Patients with appendical carcinoids are much younger, their average age at this hospital being 25 years. Several authors have called attention to the fact that the younger age of the patients with appendical carcinoids is most likely correlated with the earlier production of symptoms and consequently earlier recognition of such carcinoids.

Clinical Features.—The most common clinical manifestations of small intestinal argentaffin tumors are those of obstruction. Many carcinoids are small and symptomless; however, their tendency to produce symptoms is greater than is generally appreciated. Cooke lists 20 of the 115 carcinoids reported through 1929 as having produced symptoms of obstruction (17.4 per cent). Since then, of the 122 tumors described in the literature, including the present report, clinical manifestations were present with 38 (36 per cent). It is noteworthy to observe the increase in the numbers of clinically manifest carcinoids presented since 1929 in comparison with the relatively few cited by Cooke.

Symptoms may be produced by an obstruction of the lumen due to the polypoid nature of the growth and the tendency of these nodular growths to produce intussusception. Constriction may be caused by annular stenosing growths with an associated sclerosing fibrous reaction which frequently accompanies the tumor.

The patient may present a long history of vague gastrointestinal symptoms, with constipation or diarrhea usually a constant feature; or the onset may be sudden and acute, characterized by severe abdominal pain, nausea and vomiting. Physical examination may reveal visible peristalsis, distention, tenderness and rigidity over a given area, a movable (or fixed) mass and borborygmus. Laboratory findings are usually negative. Studies of metabolic function reveal no characteristic deviation from normal. In the presence of obstruction, roentgen find-

ings are valuable in determining the actual location of the obstruction. Since 1930 there have been 11 reports of cases in which roentgen examinations revealed small intestinal obstructions which were later found to be due to carcinoids.

In view of the fact that neoplasms so rarely involve the small intestine (5 per cent of all neoplasms of the gastrointestinal tract in the present series), and since the ileum is the site of predilection of carcinoid tumors, the presence of any growth in the ileum, especially if the growth is multiple, should suggest carcinoid tumor.

Pathology.—Carcinoids are usually small firm submucosal nodules whose cut surfaces are smooth and homogeneously yellow. They are generally freely movable beneath the surface. The overlying mucosa, although usually intact, is occasionally atrophic and infrequently ulcerated. As the nodules increase in size, they tend to bulge into the lumen as broad polypoid projections (figs. 3 *A* and 4 *A*). Such a lesion may be a factor in the production of intussusception, as in case 3. This case was similar to the one reported by McGlannan and McCleary and to the one reported by Jones. The layers of the intestinal wall can generally be differentiated.

The tumor may attain a considerable size and assume an annular form (fig. 1 *A*). There is frequently a marked fibrotic reaction leading to marked rigidity of the wall of the intestine. A large annular tumor and accompanying fibrosis may produce constriction of the lumen, even to the extent of complete obstruction.

Carcinoids may be single or multiple. Several authors have stressed the fact that multiplicity of growth is characteristic of carcinoid tumors. However, in the series reported here, only two examples of multiple carcinoid were found.

Metastases in regional lymph nodes assume the gross characteristics of the primary growth in that they are opaque, yellow and homogeneous. The nodes may be enlarged and completely replaced by tumor tissue.

The characteristic histologic picture of carcinoids presents compact cellular nests, varying from two to three cells to large sheets composed of numerous cells and separated by a variable amount of fibromuscular stroma. The cellular masses tend to assume either a solid or a pseudoglandular appearance. Both the solid and the glandular arrangement may be found within the same section, although some tumors show a preponderance of one type. In areas where the tumor infiltrates the musculature, the solid sheets of cells assume a cordlike appearance.

The cells are usually of a uniform size and polygonal. In most instances the cell boundaries are not clearly defined. The nuclei are centrally located, are spherical or infrequently ovoid and possess sharp nuclear membranes. Fine and coarse chromatin granules and numerous amitotic figures are noted. No mitoses were observed in our series.

The cytoplasm is moderate in amount, eosinophilic and fairly homogeneous, although fine, punctate granules and tiny vacuoles are contained within it.

Along the edges of many of the larger cellular aggregates are cylindric palisade cells, similar in character to the polygonal cells described in the foregoing paragraph.

The pseudoglandular structures are composed of columnar cells containing ovoid nuclei, basally situated. They have well defined cell borders, and the cytoplasm generally contains many fine granules in the infranuclear region and small vacuoles in the supranuclear area. A true lumen is not recognizable; there is often, however, a homogeneous hyalin-like eosinophilic globule in the center of the pseudogland, imparting a rosette appearance to the structure.

Two carcinoids of this series (cases 4 and 6) were characterized by cells whose nuclei were pyknotic and stained almost solid black. Transitional forms, however, could be noted between these cells and the typical polygonal cells.

Masson's sliver stain reveals numerous densely packed brownish black granules at the bases of the majority of the cells, in no way different from those found in the Kultschitzky cells of the normal intestinal mucosa.

The stroma presents a characteristic appearance, consisting of hyperplastic connective tissue and smooth muscle fibers. Masson claimed that the fibromuscular hyperplasia results from the action of the tumor cells on preexisting muscle and connective tissue. In case 2 there was noted a marked fibrohyaline mural and mesenteric sclerosing reaction with resultant constriction. The stroma usually contains numerous dilated capillaries.

A cellular reaction is not a common finding in these tumors, although infiltration of the stroma by lymphocytes, plasma cells and, to a lesser degree, polymorphonuclear leukocytes may be seen.

The smaller tumor nodules are situated in the submucosa but may extend into the mucosa and muscularis. The larger ones may involve the entire thickness of the intestine, with extension of neoplastic cells into the mesenteric fat. Lymphatic and neural invasion were noted in the cases studied here. The histologic appearance of metastases in this group was identical with that of the primary lesion except for the absence of muscular elements within the stroma.

Both circumscribed and infiltrating tumors showed similar histologic features. It was not possible from this material to evaluate what factors, if any, could be correlated with an aggressive tendency.

Malignancy.—Until recently, carcinoids were considered benign. Forbus in 1925 reported 6 cases, thoroughly reviewed the literature and called attention to the benign and harmless nature of this tumor. How-

ever, within recent year the reports of argentaffin tumors that have produced metastases have been increasing. Cooke reported that 21 in in a series of 115 cases of carcinoids of the small intestine had metastasized (18 per cent). Of the 111 cases of carcinoids reported since 1930, metastases were found in 33 (29.7 per cent). It is of interest to note that the percentage of metastasizing tumors among carcinoids which have been described since 1929 has increased. This may possibly be due to the fact that previously carcinoids were often misdiagnosed. Of the 11 cases reported here, metastases were found in 5. Thus, metastases were found in 59 of the 237 cases of small intestinal carcinoid tumors reported to date, an incidence of 24.9 per cent.

The tumor may spread by direct infiltration and by invasion of the blood stream or the lymphatics. Gáspár stated that even the smallest nests of cells possess the ability to infiltrate the muscle layer, and on this basis he holds that carcinoids should be classified as true carcinomas. Marangos expressed the belief that all carcinoids eventually become malignant through invasion of blood vessels and suggested that as the tumors get larger their tendency to metastasize becomes greater. Burckhardt claimed that, although the proliferation of cells is slow, the formation of metastases is only a matter of time, dissemination occurring mainly through the blood stream and occasionally by way of lymphatics.

In our observations there were several instances in which tumor cells could be seen penetrating the muscular wall and the serosa and infiltrating the mesentery. Tumor cells were noted within the lymphatic vessels of the primary tumors and of the metastases in the lymph nodes.

Metastases usually occur in the regional lymph nodes, the mesenteric fat and, to a lesser degree, the liver. Cooke reported 8 cases of metastasis to the liver, 10 of metatasis to the lymph nodes and 3 of extension to the serosa. Since his report, including the present group, metastases or extensions have been recorded as follows: 29 to regional lymph nodes, 10 to the liver, 9 to the mesentery, 2 to the peritoneum, 1 to inguinal and crural lymph nodes, 1 to peripancreatic and retroperitoneal lymph nodes, 1 to a testis and 1 to the dura, with compression of the spinal cord.

Mörl believes that metastases from appendical carcinoids are rare because the tumors are recognized and removed before metastasis has an opportunity to occur. Of the 21 cases of carcinoid of the appendix reported here, infiltration to the surrouding fat was noted in 1. All the others were apparently benign.

Prognosis.—The prognosis of carcinoid tumors of the small intestine is generally good. In cases in which the tumors have produced early clinical manifestations, resection is usually followed by favorable results. Of the 4 patients of this series whose carcinoids were resected, 3 are well, four years, two years and six months, respectively, after operation.

The fourth patient returned two months after operation with an abdominal mass. The mass appeared to be a local recurrence, although no definite statement can be made at this time. Following roentgen therapy, the patient is now (two and a half years after operation) entirely well; she has gained 30 pounds (13.5 Kg.) in weight, has no symptoms and shows complete disappearance of the mass.

If the tumor is symptomless for a long period of time, it may produce widespread metastases before being recognized, and then the prognosis is not as good. To emphasize the fact that carcinoids of the small intestine are far from harmless lesions, 14 deaths have been recorded since 1930, due to carcinoid tumors, their metastases or attempts at their extirpation.

Thus, of 38 patients with clinical symptoms of carcinoids reported since 1930, 13 were subjected to resection and were well for varying periods after operation. Death resulted either from the tumor or from operative intervention in 14; in 7 the results were not mentioned, 3 died of other causes, and 1 died five years after an anastomosis; this patient was symptom free after operaiton.

SUMMARY

Eleven carcinoid tumors of the small intestine are presented. Four of these produced symptoms and were resected. Three of the latter had metastasized to the mesentery and regional lymph nodes. Six of the carcinoid tumors were found post mortem. Another was an incidental finding in a surgical specimen. One of the latter 7 carcinoids had metastasized to the liver; 1, to a regional lymph node; the other 5 were benign and well localized. Histologically, the metastatic and the benign forms were structurally similar.

Many hypotheses as to the histogenesis of the carcinoid tumor have been presented. It is now generally agreed that this tumor arises from the Kultschitzky (argentaffin) cells of the intestinal mucosa.

Small intestinal carcinoids constitute 8.3 per cent of all small intestinal neoplasms and 0.02 per cent of the 47,045 specimens (both autopsy and surgical material) available for study.

Of the 237 small intestinal carcinoids (including the present 11) reported in the literature, 24.9 per cent metastasized; 24.4 per cent gave rise to clinical symptoms.

Although the prognosis is generally good, there have been 14 deaths reported since 1930 which were due either to the carcinoid tumors or to attempts at their extirpation.

In the treatment of an intestinal carcinoid tumor, resection is the method of choice. In the present series the recession of a local recurrence of such a tumor in 1 of the cases following roentgen therapy suggests that carcinoids may be radiosensitive.

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MALIGNANT CELLS IN SEROUS EFFUSIONS

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Microscopic examination of peritoneal and pleural exudates has been used for many years in attempts to arrive at the diagnosis of metastatic malignant growth. Most of the early reports on the subject deal with cases in which malignant cells were demonstrated in effusions (Quincke;[1] Dock;[2] Steiner;[3] Warren[4]). In 1917 Mandlebaum[5] described a method for embedding in paraffin blocks the sediment obtained from transudates. Smears had been used almost exclusively by the older workers; but since Mandlebaum's report the use of paraffin sections of the sediment has largely supplanted the older method (Seecof and Boetsch;[6] Bock[7]). Foord, Youngberg and Wetmore[8] conducted cellular studies on effusions, using smears and paraffin sections. They concluded that, although it was possible to suspect a malignant growth from an examination of smears, it was preferable to study paraffin sections before arriving at a final conclusion because it was easier to demonstrate cellular relationships by this method.

Zemansky[10] reported the histologic study of sediment from 89 abdominal and pleural effusions in cases in which by subsequent study a definite diagnosis was made by the results of roentgenologic examination, biopsy or necropsy. He used paraffin sections, assuming that fragments of tissue often were destroyed by the process of smearing. In 87 per cent of the cases in which he reported the thoracic and abdominal fluids as showing neoplastic cells he was correct, and in 41 per cent of those in which he reported the fluids as not indicating presence of a neoplasm he was correct. There were 34 serous fluids in the examination of which a positive diagnosis of malignancy had been made; in

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5 cases the diagnosis subsequently was disproved, and in each instance there was a history of long-standing effusion either from cirrhosis of the liver or from congestive heart failure. The criteria for malignancy used by Zemansky [10] were fragments of tumor, multiple groups of deeply stained cells and the finer cellular changes, such as irregularity of the cell, a large nucleus and mitotic figures. Karp [11] found mitotic figures in cells of mesothelial origin, although others (Graham [12]) felt that it was doubtful whether they occurred in other than neoplastic cells. Quensel [13] made a thorough study of cells in effusions by staining the sediment in the fresh state with methylene blue and sudan. He was able to distinguish between neoplastic cells and endothelial cells.

Graham felt that the clumps of cells frequently found in serous fluids often were caused by fusion of the cells of the exudate. Goldman [14] observed that mesothelial cells frequently were clumped in serous fluids and could be mistaken for malignant cells. Merklen, Waitz and Kabaker [15] studied smears of serous effusions stained by the May-Grünwald-Giemsa stain and decided that giant cells in fluids were caused by amitotic division of the cells. Quensel,[16] Karp [11] and Foot [17] applied the nuclear-nucleolar ratio in the diagnosis of malignancy in cells found in effusions and reported favorably on its practical importance in distinguishing between benign and malignant cells.

TECHNIC

This report is based on a microscopic study of the sediment obtained from 97 peritoneal and pleural fluids. Excepting purulent effusions, which usually were not studied cytologically, this group represents a consecutive series of fluids examined in this laboratory. The technic which was used in examination of these specimens was simple. From 20 to 40 cc. of the fluid was centrifuged for fifteen minutes at approximately 1,800 revolutions per minute. When possible, the last portion of fluid which was withdrawn from the cavity was chosen, although frequently a choice could not be made. The fluid was centrifuged immediately after removal because the clot which forms after standing interferes with this method of examination. The centrifugate was smeared rather quickly on clean glass slides and was allowed to dry in air. The slides were immersed in solution of formaldehyde U. S. P. (diluted 1 to 10) for twelve or more hours. The smears then were stained with hematoxylin and eosin, carried through the alcohols, carbol xylene and xylene and mounted in Canada balsam.

In each instance the diagnosis was made without knowledge of the clinical aspect of the case. This tended to increase caution in making a diagnosis of malignancy. The clinical records of the cases then were obtained, and relevant data were noted.

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RESULTS

Fluids in Which Malignant Cells Were Found.—Malignant cells were found in 34 fluids removed from 30 patients. In 11 cases the source of the fluid was peritoneal and in 19 pleural.

Fragments of neoplastic tissue offer the most reliable evidence for the diagnosis of malignancy from a study of serous effusions. Portions of tumor could be demonstrated in only 2 cases in this series. Both specimens were ascitic fluid, and a definite diagnosis of adenocarcinoma could be made on the basis of the glandular arrangement. In the remainder of the fluids the neoplastic cells were found singly or in small groups, and in regard to these specimens the diagnosis was made on morphologic observation of the individual cells (fig. 1 *A*). The malignant cells were recognized by their large nuclei and their prominent nucleoli. Usually there was considerable variation in the size of the neoplastic cells. This variation, together with the irregularity of the cell, could not be depended on too much, because of the degenerative changes which result from the cell's being in fluid for a variable length of time. Mitotic figures were found in the malignant cells in 13 cases. In few cases were they numerous. Usually it was necessary to search carefully to find them (fig. 1 *B* and *C*).

A frequent observation in the cells was the appearance of the nucleus at the side of the cell (fig. 2*A*). Twenty-three of the fluids in this group showed this change; both endothelial and malignant cells were involved in the process. This change was not seen in any other type of cell. It suggested the presence of intracellular mucus; however, the mucicarmine stain failed to indicate the presence of this substance as such in all except a single case. The true character of the cell usually could be determined by studying the nucleus, which remained relatively unchanged.

In 23 cases there was clumping of the malignant cells (fig. 1*C*). At first glance this appearance suggested neoplastic giant cells. However, in a number of fluids in which malignant cells could not be demonstrated, cells of unquestioned endothelial origin gave a similar appearance.

Endothelial cells, lymphocytes and erythrocytes were found most frequently associated with malignant cells in fluids. In order to compare normal endothelial cells with those found in cases of serous effusion, the peritoneal surface of stomachs which had been removed surgically for benign conditions was scraped with a knife and the cells were smeared on slides. The smears were then treated as were those obtained from sediment of fluids. Endothelial cells obtained in such a fashion had centrally placed small regular nuclei with considerable cytoplasm (fig. 2*B*).

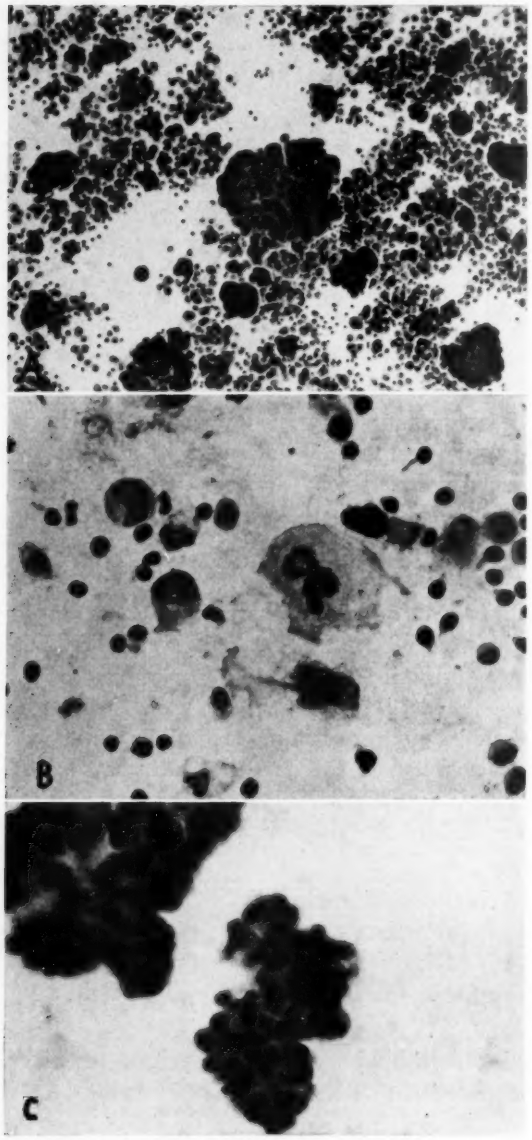

Fig. 1.—*A*, sediment from pleural effusion; hematoxylin and eosin; × 105.5. The neoplastic cells are found both singly and in clumps and are in contrast with the benign endothelial cells. *B*, sediment from pleural effusion; hematoxylin and eosin; × 327. The neoplastic cells can be recognized by the large nuclei. A mitotic figure is also shown. *C*, sediment from peritoneal effusion; hematoxylin and eosin; × 254.5. Two clumps of neoplastic cells are shown; the cells are large and irregular and contain several mitotic figures.

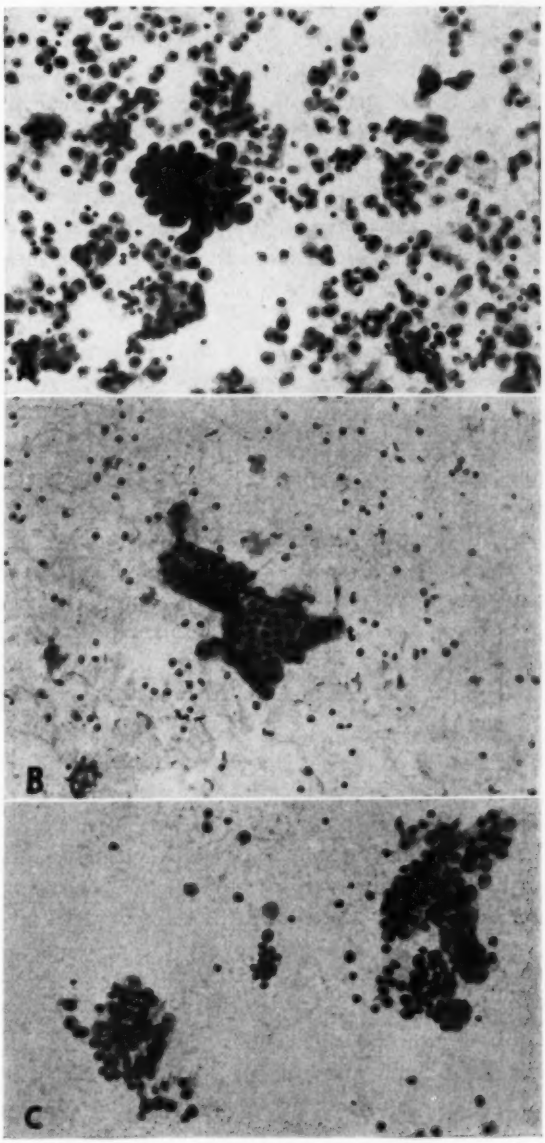

Fig. 2.—*A*, sediment from peritoneal effusion; hematoxylin and eosin; × 147. The malignant cells and endothelial cells have eccentric nuclei. The neoplastic cells occur for the most part singly, but there is one clump. *B*, smear of peritoneal cells from a stomach removed for benign gastric ulcer; hematoxylin and eosin; × 139.3. The cells are regular and contain small nuclei approximately twice as large as the surrounding lymphocytes. *C*, sediment from peritoneal effusion; hematoxylin and eosin; × 143.2. The endothelial cells are swollen and have eccentric nuclei. Tumor cells are not present.

An attempt was made to reproduce the changes which occurred in the malignant and endothelial cells by which the nuclei assumed their eccentric position. Cells were scraped from the peritoneal surface of fresh surgically removed specimens. Some were placed in 0.2 per cent saline solution, another portion in 0.85 per cent saline solution and the remainder in 1.5 per cent saline solution. These were kept at room temperature for varying periods up to twelve hours. The fluids then were centrifuged, and the centrifugate was treated in a manner similar to that used for smears obtained from the sediment of serous effusions. Many of the endothelial cells which had been placed in all three solutions had eccentric nuclei. This change was, however, more severe in the hypotonic (0.2 per cent) saline solution. Lymphocytes and polymorphonuclear leukocytes did not show this change.

In 18 cases in which the diagnosis of malignant growth was made from microscopic examination of effusions, it was impossible to confirm the impressions by necropsy or biopsy. In 12 cases tissue was available for study (table). All the malignant tumors in this group were adenocarcinoma. In those cases in which confirmation of the diagnosis of malignant growth could not be obtained, there was clinical or roentgenologic evidence suggesting the presence of a malignant neoplasm in 12. In 2 of the remaining cases, the biologic test for tuberculosis by the use of guinea pigs was negative. In 4 of the remaining cases, there was no evidence that the diagnosis of malignant growth was wrong, although definite proof was lacking.

Fluids in Which Malignant Cells Were Not Found.—In 63 fluids obtained from 41 patients malignant cells could not be demonstrated. Of these effusions, 48 were pleural and 15 abdominal. The endothelial cell was the most frequent type of cell encountered in this group of fluids. As a rule, it was easy to distinguish between these and malignant cells, but in certain cases, particularly those in which the effusion had been present over a long period of time, it was found that the endothelial cells were swollen and irregular. As has been stated, frequently endothelial cells were found assuming the formation of signet ring cells (fig. 2*C*). Clumping of endothelial cells was found in 22 fluids in which malignant cells could not be recognized. Other types of cells which were found in the smears from this group were lymphocytes, erythrocytes, neutrophils and eosinophils. It was always easy to distinguish these cells from malignant ones.

The clinical records of the 41 cases in which malignant cells were not found in the effusions were divided into three groups: those in which a definite diagnosis of malignant growth was made, based on the results of biopsy or necropsy, those in which it could be shown that the effusion was owing to causes other than a neoplasm, and those in which the diagnosis was indeterminate. There were 13 cases in the first group,

10 of these being cases of carcinoma. Malignant neoplasms were not responsible for the development of effusion in 17 cases. In the majority of these the effusion was due to tuberculosis, cirrhosis of the liver or an inflammatory condition. In 11 cases it was impossible to arrive at a definite diagnosis.

Cases of Proved Malignant Neoplasm in Which Malignant Cells Were Found in Pleural or Peritoneal Fluids

Case	Sex	Site of Fluid	Predominant Cell Other Than Malignant Cell	Clumping	Positive Roentgen Data	Report of Biopsy or Necropsy
1	F	Peritoneum	Lymphocyte	Present	.....	Biopsy; adenocarcinoma primary in ovary
2	M	Pleura	Lymphocyte and endothelial	Present	.....	Necropsy: adenocarcinoma involving liver, pleura and so forth (primary?)
3	F	Pleura	Lymphocyte	Present	Destruction of third rib and sternum	Adenocarcinoma of right breast with involvement of axillary nodes
4	F	Pleura	Lymphocyte	Present	.....	Biopsy: adenocarcinoma involving left axillary node, primary in breast
5	F	Peritoneum	Endothelial	Present	.....	Biopsy: adenocarcinoma primary in ovary
6	F	Peritoneum	Lymphocyte	Present	.....	Biopsy: adenocarcinoma primary in breast
7	F	Peritoneum	Lymphocyte	Pieces of tissue	.....	Biopsy: adenocarcinoma of right ovary with peritoneal implants; patient died; no necropsy
8	F	Peritoneum	Endothelial	Present	.....	Biopsy: adenocarcinoma of right ovary with peritoneal involvement
9	F	Peritoneum	Endothelial	Present	Increased density of right pleura, (?), malignant growth	Necropsy: adenocarcinoma of left ovary with peritoneal implants
10	M	Peritoneum	Endothelial	Absent	Carcinoma in middle portion of stomach	Adenocarcinoma of stomach (linitis plastica) with peritoneal involvement
11	M	Peritoneum	Lymphocyte	Pieces of tissue	.....	Colloid adenocarcinoma (positive for mucus)
12	M	Pleura	Endothelial	Present	Extensive infiltration of right lung	Necropsy: adenocarcinoma of lung with extensive metastasis to pleura and other tissues

COMMENT

The study of cells found in serous effusions is a valuable procedure and should be used in cases in which the presence of a malignant neoplasm is suspected. Our results verify the observation made by Zemansky,[10] namely, that the diagnosis of a neoplasm based on the observation of neoplastic cells in the sediment obtained from fluids carries a high degree of accuracy, whereas a report that the sediment shows no such cells is of much less value.

Contrary to contemporary opinion, we have found that smears of the sediment fixed in a solution of formaldehyde and stained with hematoxylin and eosin offer a satisfactory method for the study of cells in serous fluids. It is impossible, however, for us to compare smears with sections made by embedding the sediment in paraffin blocks, because the latter method was not used on any of the material in this series.

The diagnosis of malignant cells is made best by finding large cells with large nuclei and nucleoli. Mitotic figures are a valuable aid in making the diagnosis. However, it is usually difficult to find mitoses. Since the cells are often in a degenerated state, pyknosis and karyorrhexis must be eliminated, for degenerated forms of cells at times simulate mitotic figures rather closely. Mitotic figures were not found in endothelial cells, although they have been described (Karp [11]).

Clumping of the cells was found in the sediment in approximately half of the effusions examined. Clumping was observed in fluids that contained only benign and in those that contained both benign and malignant cells. It appears to be owing to a fusion of the cells rather than to multipolar mitosis, as has been stated by Graham, and is of little diagnostic importance, because both endothelial and neoplastic cells can be involved in the process. The eccentricity of the nuclei of certain cells in serous effusions is also of little diagnostic value. Such a change is found not only in neoplastic cells but also in endothelial cells.

SUMMARY

Examination of the sediments obtained from effusions is of definite value in cases in which malignant growths involving serous cavities are suspected.

Smears made from the sediments of fluids are as efficacious in studying cellular detail as paraffin sections. Cells with eccentric nuclei and clumped cells are of little value when one is determining the nature of serous effusions.

When malignant cells can be identified definitely in serous effusions, the diagnosis of malignant growth carries a high degree of accuracy.

NEGATIVE CHEMOTROPISM IN LEUKOCYTES

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AND

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PHILADELPHIA

The outcome of an infection—whether recovery or death—may depend on the ability of the leukocytes to display positive chemotropism, i. e., to move toward the infecting bacteria, thus making possible phagocytosis and destruction of the invading organisms. If leukocytes are unable to approach bacteria, or if they are actually repelled so that they move in the opposite direction, i. e., display negative chemotropism, phagocytosis cannot occur. It is therefore of interest to ascertain under what conditions, if any, leukocytes exhibit negative chemotropism. A survey of the literature shows few instances in which under experimental conditions leukocytes are actually repelled by bacteria or substances of any kind, and while the terms "negative chemotropism" and "negative chemotaxis" are often used, they indicate as a rule merely absence of attraction rather than repulsion.

However, a repelling effect was observed by Jochims [1] to be exerted by high concentrations of isotonic calcium chloride and barium chloride and by strongly acid solutions. Mouse muscle was found by Grand and Chambers [2] to prevent the approach of chicken leukocytes. Probably this effect also is an instance of negative chemotropism.

The purpose of the present paper is to record the results of experiments in which definite repulsion of leukocytes was induced. While studying the chemotropic action of fractions of hemolytic streptococci adsorbed on kaolin,[3] we observed the majority of rabbit leukocytes to move away from untreated kaolin used in control preparations (unpub-

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This investigation was aided by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

1. Jochims, J.: Arch. f. d. ges. Physiol. **216**:611, 1927.

2. Grand, C. G., and Chambers, R.: J. Cell. & Comp. Physiol. **9**:165, 1936.

3. Dixon, H. M.; McCutcheon, M., and Czarnetzky, E. J.: Am. J. Path. **13**:645, 1937.

lished data).[4] The chemotropic effect of kaolin has now been reinvestigated in a new series of experiments.

NEGATIVE CHEMOTROPISM TOWARD SILICATES

Method.—Kaolin is a fine white powder prepared from fullers' earth, consisting essentially of aluminum silicate which has been purified by treatment with hydrochloric acid. In these experiments the powder was washed twice in redistilled water, and then a minute drop of water containing particles of kaolin was transferred with a platinum loop to a glass slide. After drying, the particles formed a circular clump about 0.5 mm. in diameter.

Leukocytes were obtained by injecting 150 cc. of isotonic sodium chloride into the peritoneal cavity of a rabbit. The fluid was withdrawn after about four hours, and the cells after being concentrated by light centrifugation were resuspended in plasma of the same animal. A drop of the cell suspension was placed on a cover slip and lowered on the kaolin, so that the cell suspension spread between the slide and the cover slip. The preparation was sealed to prevent evaporation and examined with the microscope at 37.5 C. By means of a drawing ocular, the image of a microscopic field containing a portion of the kaolin and a number of leukocytes was projected on paper, and the position of each leukocyte was recorded at intervals of a minute for ten minutes.

The value of the chemotropism was found for each microscopic field as follows: The initial distances of a number of leukocytes (usually from 10 to 20) from the edge of the clump of kaolin were obtained and added. Similarly, the distances from the kaolin at the last observation were obtained for the same cells and added. The difference between these sums was divided by the sum of the minutes of observation, giving the value of the chemotropism in microns per minute.[5] This value is positive if the cells on the average move toward the substance tested, negative if away from it, and zero if they move neither toward nor away from it. If in a series of such experiments the mean value is significantly above or below zero, the leukocytes are regarded as having shown positive or negative chemotropism, respectively, while if the mean value is not significantly different from zero, the cells have shown no chemotropic reaction.

In this way the values for the chemotropism in eighteen microscopic fields were found, one or two fields being measured in each experiment. The mean value of the chemotropism was -3.2 microns per minute, and the standard error of the mean was ± 0.79. The difference between the mean and zero is significant, and we conclude that the leukocytes were definitely repelled by kaolin.

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4. D. Silverman (Arch. Path. **25**:40, 1938) reported that kaolin induces a similar negative response in human leukocytes, though W. B. Wartman (ibid., to be published) observed that human leukocytes are neither attracted nor repelled by this substance.

5. The computation may be illustrated by reference to figure 1, in which the paths of 10 leukocytes are represented. At the first observation the sum of the distances of these cells from the silicate was 1,402 microns; at the last observation, 2,133 microns. The difference is 731 microns. The sum of the minutes of observation is 64. The value of the chemotropism is $-731/64$, or -11.4 microns per minute.

Since negative chemotropism was produced by kaolin, the question arose whether other silicates would produce similar effects. At the suggestion of Dr. G. H. A. Clowes, another preparation of purified fullers' earth was tested [6] by the method described. This silicate also was found to induce negative chemotropism. It was decidedly more effective than kaolin. The result of a representative experiment is shown in figure 1, in which the path of each leukocyte was recorded for ten minutes, or for a shorter time if the cell left the field. It is seen that all the cells moved away from the silicate, most of them traveling more or less directly away. The value of the chemotropism in this field is — 11.4 microns per minute, a high negative value. A negative reaction was found in all of 18 similar preparations. The mean chemotropic value was — 7.1 microns per minute.

The fact that acids were used in the preparation of both kaolin and this silicate suggested the possibility that all the acid might not have been removed and that this might be responsible for the repelling effect. This possibility was tested by placing the silicate for half an hour in a concentrated solution of sodium hydroxide. The alkali was then removed by repeated washing with distilled water. In this way any free acid would be neutralized.

To test the effect of this treatment with alkali, untreated silicate and silicate treated with sodium hydroxide were placed on the same slide about 5 mm. apart and included in the same preparation. Microscopic fields adjacent to the two clumps of silicate were observed alternately in a series of preparations. In all, eight pairs of microscopic fields were observed. The mean chemotropic value for untreated silicate was — 7.0 microns per minute; for silicate treated with sodium hydroxide it was — 6.0. The mean difference and its standard error were 1.0 ± 0.99 micron per minute, so that the difference between untreated silicate and silicate treated with sodium hydroxide is not significant.[7] It is concluded that the negative chemotropism produced by silicate is not due to acid substances.

The next step was to consider aluminum silicate as composed of aluminum oxide and silicic acid and to test these substances separately, to find out if possible in which fraction the repelling effect lies. Aluminum oxide exerted no significant chemotropic effect; the mean

6. This fullers' earth is marketed as Lloyd's Reagent by Eli Lilly & Company, who supplied a sample. We are advised that this is a refined white English fullers' earth, originally consisting mostly of aluminum silicate; most of the base is dissolved by leeching with acid, which is then removed by washing. Hereafter we shall refer to this preparation as a silicate.

7. For the statistical treatment of such small samples the reader is referred to L. H. C. Tippett (The Methods of Statistics, London, Williams & Norgate Ltd., 1931) and to D. Mainland (The Treatment of Clinical and Laboratory Data, London, Oliver & Boyd, 1938).

value was + 1.2 microns per minute. With silicic acid a weakly negative value, — 1.8 microns per minute, was obtained, which, while significant, is far less than the negative effect produced by the silicate. Evidently further work is needed to explain the repelling effect of the silicate, and such experiments are in progress.

The results of the experiments described thus far have been brought together in the accompanying table.

Chemotropic Response of Polymorphonuclear Leukocytes to Aluminum Silicate

Substance Tested	Microscopic Fields	Mean Value of Chemotropism and Standard Error, Microns per Minute*
Kaolin	18	−3.2±0.79
Silicate (a refined white English fuller's earth)	18	−7.1±0.39
Silicate + sodium hydroxide	8	−6.0±0.84
Aluminum oxide	11	+1.2±0.81
Silicic acid	11	−1.8±0.59

* The values for kaolin, silicate and silicate plus sodium hydroxide are significantly negative; silicic acid also is probably significantly negative, since the mean is about three times its standard error, but the value for aluminum oxide is not significantly different from zero (absence of chemotropism), since the mean is less than twice its standard error. An average of 13 leukocytes was observed in each microscopic field.

CHEMOTROPIC RESPONSE TO HEMOLYTIC STREPTOCOCCI

Having shown that negative chemotropism (actual repulsion) occurs and that it may be demonstrated without fail under the conditions stated, it was interesting to inquire whether any bacteria produce negative chemotropism, for this would be of considerably more practical importance.

The answer is qualified: We have found no clearcut example of negative chemotropism induced by bacteria, but some evidence of a repelling effect has been found in the reaction of leukocytes to a strain of beta hemolytic streptococci.

To make this effect clear, the chemotropic response to another organism, Staphylococcus albus,[8] is shown first (fig. 2). It is seen that all the leukocytes moved toward the bacteria, and that they continued to do so until they came in contact with them.

A very different reaction is shown to Streptococcus haemolyticus, as seen in figure 3. It is seen that each cell at first moved toward the bacteria and then turned away. One cell then traveled in the opposite direction until it left the microscopic field. Other cells moved alternately toward and away from the bacteria but failed to come in contact with them.

8. The reaction of leukocytes to Staph. albus has been discussed more fully in earlier papers (Dixon, H. M., and McCutcheon, M.: Proc. Soc. Exper. Biol. & Med. **34**:173, 1936; **38**:378, 1938).

The chemotropism in the presence of these streptococci, computed according to the method described, was usually positive during the first ten minutes of observation. Thereafter the chemotropism tended toward zero; i. e., the leukocytes moved on the average neither toward nor away from the bacteria. This behavior may be interpreted either as random motion, due to absence of attractive substances, or as a complicated reaction to both attracting and repelling substances present at the same time.[9] Which of these interpretations is correct is at present uncertain.

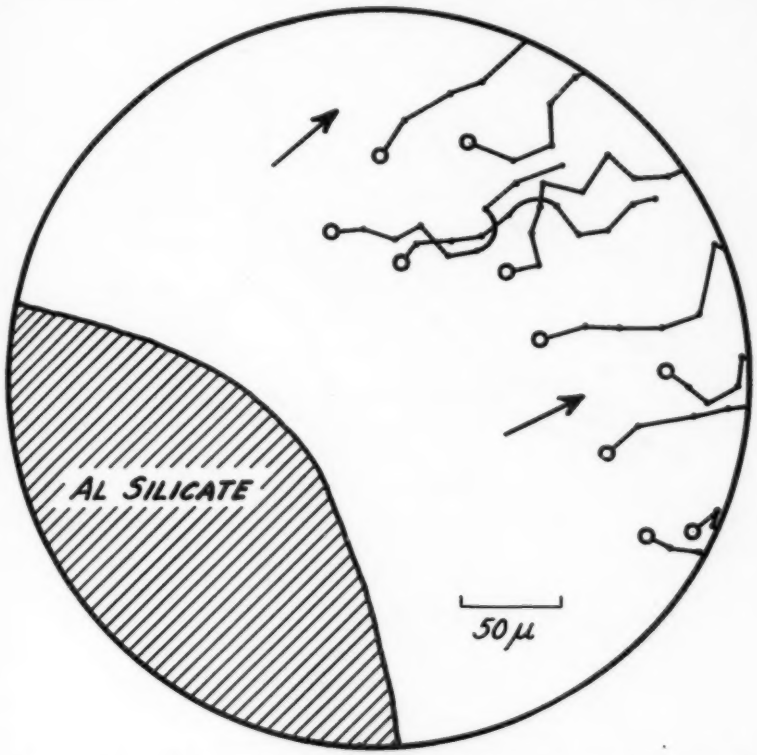

Fig. 1.—The reaction of polymorphonuclear leukocytes to aluminum silicate (a refined white English fullers' earth); a camera lucida record of the paths of 10 cells. Circles represent the position of the leukocytes when first observed, and dots show successive positions at intervals of a minute. It is seen that all the leukocytes moved away from the silicate.

But in a number of experiments, as illustrated in figure 3, leukocytes appeared unable to reach the bacteria.[10] They approached until they

9. A somewhat similar reaction is shown by human leukocytes to the pathogenic yeast Torula histolytica (McCutcheon, M., and Dixon, H. M.: Arch. Path. **21**:749, 1936).

10. The detailed results of these experiments will be published later.

were 30 or 50 microns distant from the bacteria and then appeared to turn aside. This "avoiding reaction" was shown in some experiments but not in others. There was a striking difference in the numbers of leukocytes that collected about the clumps of staphylococci and streptococci. The former, after half an hour were surrounded by a dense wall of leukocytes, whereas about the streptococci there were as a rule only a few small collections of leukocytes here and there. It is well known that in vivo there is often a corresponding difference in the numbers of

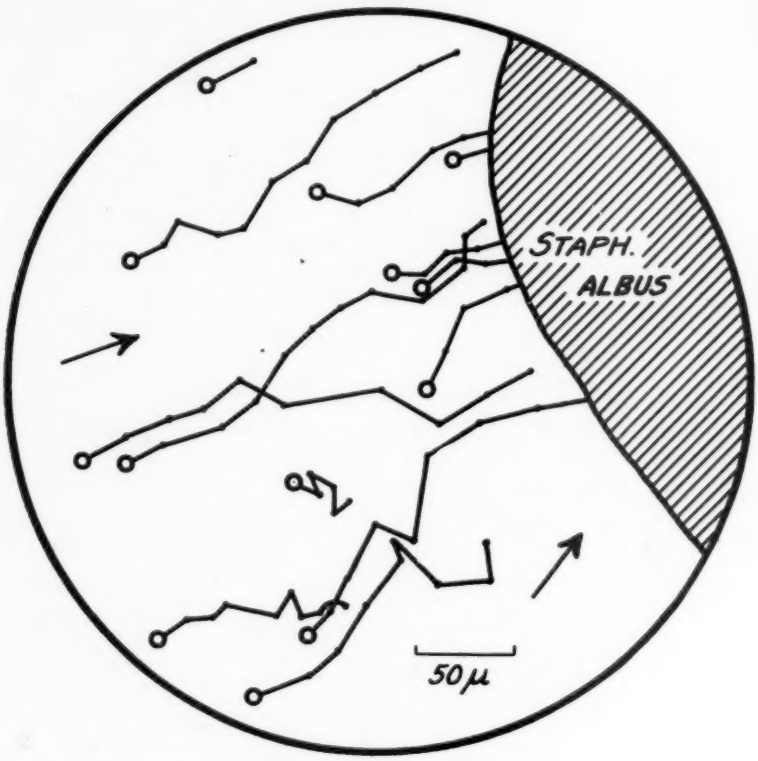

Fig. 2.—The reaction of polymorphonuclear leukocytes to Staph. albus. Part of the clump of bacteria is shown to the right. The paths of 13 leukocytes were recorded for ten minutes, or until the cells reached the bacteria. All the leukocytes moved toward the bacteria.

leukocytes found in tissues infected with staphylococci and streptococci, respectively. The tendency of streptococcic infections to spread may be due in part to the inadequate response of leukocytes, for if leukocytes are not attracted to the bacteria, and especially if leukocytes are repelled by the bacteria, phagocytosis with subsequent destruction of bacteria cannot occur.

In regard to phagocytosis of bacteria in our experiments, it was repeatedly observed that the few leukocytes that succeeded in reaching the streptococci phagocytosed them freely. This observation suggests that the phagocytic index alone is not an adequate measure of the ability of leukocytes to combat an infection. In the infection in vitro as set up in our experiments the fault was not with phagocytosis but with chemotropism; the leukocytes were able to phagocytose, but most of them were prevented from doing so either because the streptococci did not

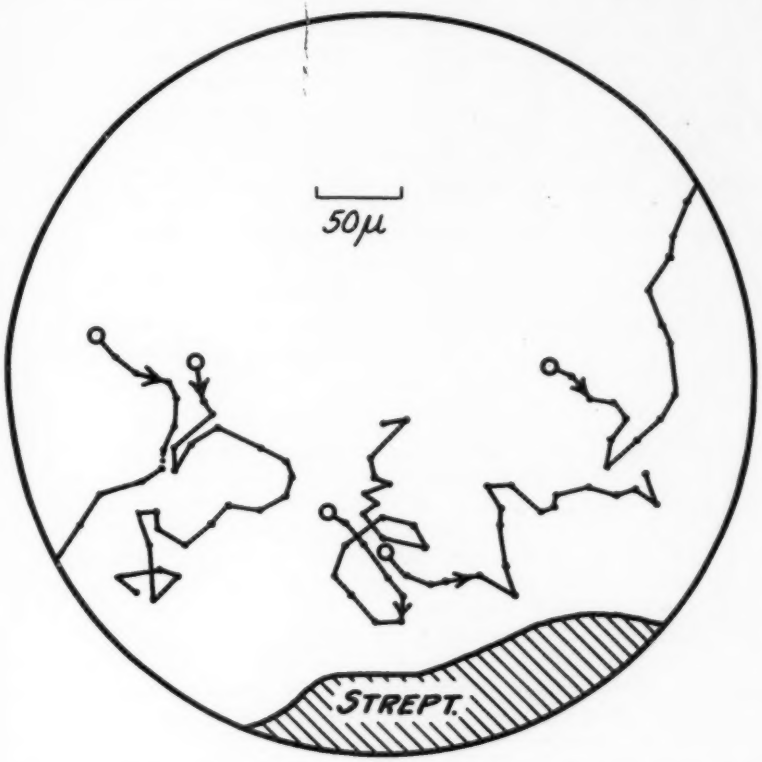

Fig. 3.—The reaction of polymorphonuclear leukocytes to Strep. haemolyticus. To avoid confusion, the paths of only 5 leukocytes are reproduced. The leukocytes first approached and then moved away from the bacteria. The time of observation was thirty-one minutes.

produce substances that attracted leukocytes (absence of chemotropism) or because they gave off repelling substances (negative chemotropism).

SUMMARY

We have investigated the question whether leukocytes exhibit negative chemotropism, by which is meant not merely absence of attraction

but actual repulsion by some substance. It was found that a silicate[6] regularly induces negative chemotropism in rabbit polymorphonuclear leukocytes in vitro. The repelling effect was found not to be due to acid substances, since it persisted after the silicate was treated with alkali, nor was it due to aluminum base. The anion, silicic acid, by itself exerted only a weakly repelling action, so that further study is necessary to explain the repelling effect of the silicate. Similar but weaker negative chemotropism was induced by a related substance, kaolin.

In the presence of a strain of beta hemolytic streptococci, rabbit leukocytes were attracted by the bacteria for a few minutes; thereafter the cells moved toward the bacteria in some experiments but showed an avoiding reaction in others. Only a few leukocytes succeeded in reaching the bacteria, but these few phagocytosed the bacteria freely. It is likely that such a deficient chemotropic reaction may account in part for the poor leukocytic response in certain streptococcic infections.

PITUITARY FIBROSIS WITH MYXEDEMA

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AND
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In 1914 Simmonds [1] described a syndrome of severe cachexia, premature senility, loss of hair, pigmentation of the skin, amenorrhea and weakness. Patients with this syndrome showed destruction of the anterior lobe of the pituitary, fibrosis of the thyroid and ovaries, atrophy of the adrenals, parathyroids and endometrium, and microsplanchia. About 75 cases of this syndrome have been reported. Silver's [2] recent article on the subject is an excellent review. One of the most striking features of the syndrome is the extreme grade of cachexia, without which the condition is not diagnosed.

We have recently studied a case that was believed clinically to be one of myxedema but which showed pathologically all the findings seen in Simmonds' disease except the cachexia.

REPORT OF CASE

A 48 year old Italian housewife entered the hospital Dec. 1, 1936, complaining of pain in the legs and left wrist of two months' duration.

Ten years before admission, when she was 38 years old, she had a miscarriage at five months in her first pregnancy. Within the next year she had a sudden onset of amenorrhea without attendant symptoms. Prior to this time she had had normal, regular catamenia. After this time she had no bleeding. During the ten years prior to her admission to the hospital she failed in general health, and there was vague gradual development of the symptom complex to be described. She stated that her skin grew progressively drier and coarser over a period of nearly ten years and that over a period of about two years it had shown scaling. For about five years she noted slow gradual loss of strength and enterprise, development of a placid disposition and rare bouts of nervousness and irritability. Four years before she entered the hospital she first noted severe headache, most marked on the left side of her head, which had no definite relation to eyestrain, food or other factor. During the past year she noticed loss of appetite and restricted her diet largely to carbohydrates. She also noticed that the constipation with which she had been troubled for years was becoming more marked. Two months before examination she first noticed definite pain in the bones, joints and muscles

The expenses for the biologic assays were met by the Proctor Fund of the Harvard Medical School.

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1. Simmonds, M.: Deutsche med. Wchnschr. **40**:322, 1914.
2. Silver, S.: Arch. Int. Med. **51**:175, 1933.

of her left arm and legs. For many years she had had bouts of pain in the spine, arms and legs, apparently mild. She did not describe these attacks accurately but attributed them to her insomnia and loss of strength.

"Rheumatism" had been present intermittently since the age of 15.

She was well developed, well nourished and in no discomfort. The skin over all the extremities was atrophic, dry and scaly; also, to a less degree, that over the trunk. The hair was dry and coarse. The gums were inflamed. There was questionable lichen planus of the mouth. The heart sounds were of only fair quality. The cardiac rate varied from 54 to 58 beats per minute. The blood pressure was 160 systolic and 100 diastolic. Pelvic examination showed an atrophic cervix.

The urine was normal. The blood showed 4,500,000 red cells and 5,500 white cells per cubic millimeter. The hemoglobin content was 85 per cent. The differential white cell count showed 67 per cent polymorphonuclears. The Hinton test was negative. The basal metabolic rate was —28 per cent. The blood showed cholesterol 374, calcium 9.85 and phosphorus 4.2 mg. per hundred cubic centimeters, and phosphatase 6.44 Bodansky units. Analysis of the gastric content showed free hydrochloric acid. The nonprotein nitrogen of the blood serum was 18 mg. per hundred cubic centimeters. A lumbar puncture gave negative results. An electrocardiogram showed normal rhythm, a rate of 72 and tracings consistent with myxedema.

The patient was believed to have myxedema, and thyroid extract was administered by mouth. She became nauseated and vomited. On the fourteenth day she was given thyroxin U. S. P. intravenously. Her drowsiness increased, and on the nineteenth day a psychosis developed. All the extremities were pseudospastic, although no other neurologic signs were present. The reflexes were active. The following day she had a convulsion, which lasted about four minutes. She refused to speak, move or eat, and on the twenty-second day her temperature became elevated. During this period the signs of myxedema disappeared, the temperature remained elevated between 102 and 105 F., rales developed in both lungs, and death occurred on the thirtieth day.

Autopsy.—The pertinent gross observations were as follows:

The body was that of a small, well developed and well nourished 48 year old woman weighing approximately 115 pounds (52.1 Kg.). The skin was only slightly roughened, especially over the arms, and was not darkened or pigmented. The mucous membrane on the inner aspects of the cheeks was grayish white, glistening and smooth. The abdominal subcutaneous fat was bright yellow and measured 4 cm. in thickness. The muscles appeared normal. The thyroid was small, weighing 4.8 Gm. The surface was grayish pink and smooth. The tissue cut with increased resistance, and the cut surface was pinkish gray and fibrous, with no colloid definitely discerned. Each lobe measured approximately 3 by 2 by 0.7 cm. Three normal-sized parathyroid glands were found. The lungs showed numerous foci of bronchopneumonia. The heart was small, weighing 175 Gm. The spleen weighed 125 Gm. The adrenals were very small, together weighing 5 Gm. On section there was marked narrowing of the cortex and medulla, the former measuring approximately 1 mm. The kidneys weighed 150 Gm. and were normal. The uterus was small and atrophic. The myometrium was narrow, measuring from 6 to 7 mm. in thickness. The endometrium was grayish white, smooth and very thin. Both ovaries were atrophic, together weighing 4 Gm.; on section no follicles were visible. The brain weighed 1,200 Gm. and showed normal convolutional markings. The stalk of the pituitary was normal. The whole gland, however, which was removed with the surrounding dura, was

markedly atrophic. The gland, excluding the stalk, measured approximately 5 by 5 by 2 mm. A definite division into lobes could not be made out. The sella turcica appeared slightly shallow.

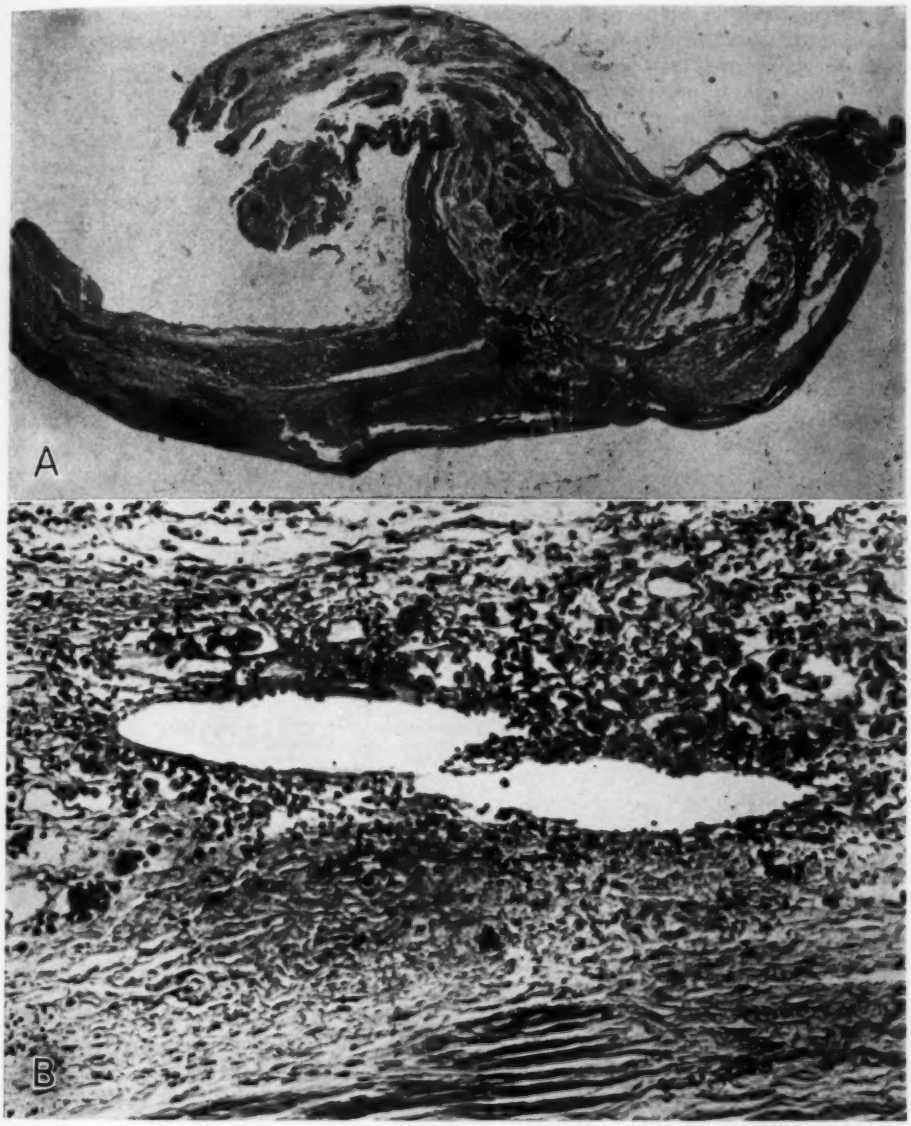

Fig. 1.—*A*, cross section of the whole pituitary, showing the extreme fibrosis of the anterior lobe. *B*, higher magnification of a portion of the anterior lobe, showing the lymphocytic infiltration around some of the epithelial cells.

Microscopic Examination.—Serial sections of the whole pituitary gland were made and stained in two ways: (1) with hematoxylin and eosin and (2) by a modification of the Mallory aniline blue stain for connective tissue which consisted in using hematoxylin before the regular staining process. The sections (fig. 1) showed that almost all of the anterior lobe had been replaced by dense fibrous tissue in which were scattered a few remaining isolated groups of small epithelial cells, often surrounded by a lymphocytic infiltration. The posterior lobe was possibly smaller than normal but showed no evidence of fibrosis. The vessels disclosed no diagnostic abnormality.

Several sections through various parts of both lobes of the thyroid (fig. 2) showed a similar process. There were broad interlacing bands of fibrous con-

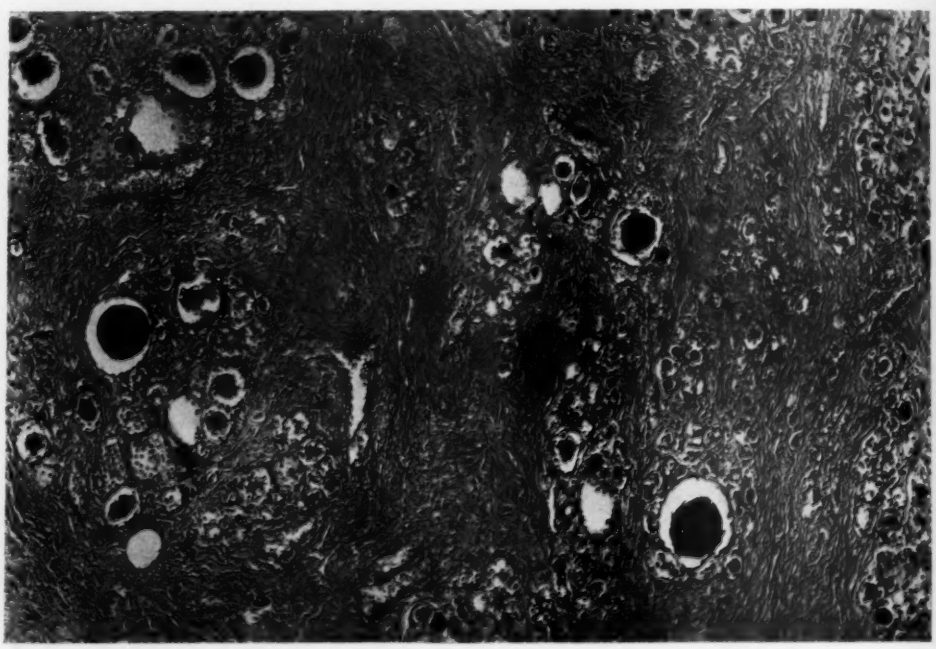

Fig. 2.—A section of thyroid showing the extreme grade of fibrosis.

nective tissue, in between which were very small thyroid follicles, some empty and others filled with colloid. There was no cellular infiltration or any evidence of acute or chronic infection. The striking feature was the extensive fibrous stroma. The larger vessels showed fibrous intimal thickening but no thrombosis.

Except for its small size, the adrenal was normal. The cells in both the cortex and the medulla were normal. In the center of the medulla, in one small area there was a slight lymphocytic infiltration with fibrosis.

The pancreas was normal. Both ovaries showed complete atrophy; there was no evidence of activity. Numerous corpora albicantia were found.

The uterus had a completely atrophic endometrium. Only an occasional gland was seen, and these were very small and showed no evidence of activity.

Approximately nine tenths of the parathyroid glands was composed of fat cells (fig. 3). The remaining parathyroid cells were normal chief cells with only an

occasional oxyphilic cell. This extreme fat replacement certainly evidenced atrophy. There was no cellular reaction or fibrosis.

Except for slight bronchopneumonia and lichen planus of the mouth, the other organs were normal.

REVIEW OF THE LITERATURE

In the literature we have been able to find records of only 6 cases similar to the case reported here. Some of these have been included in

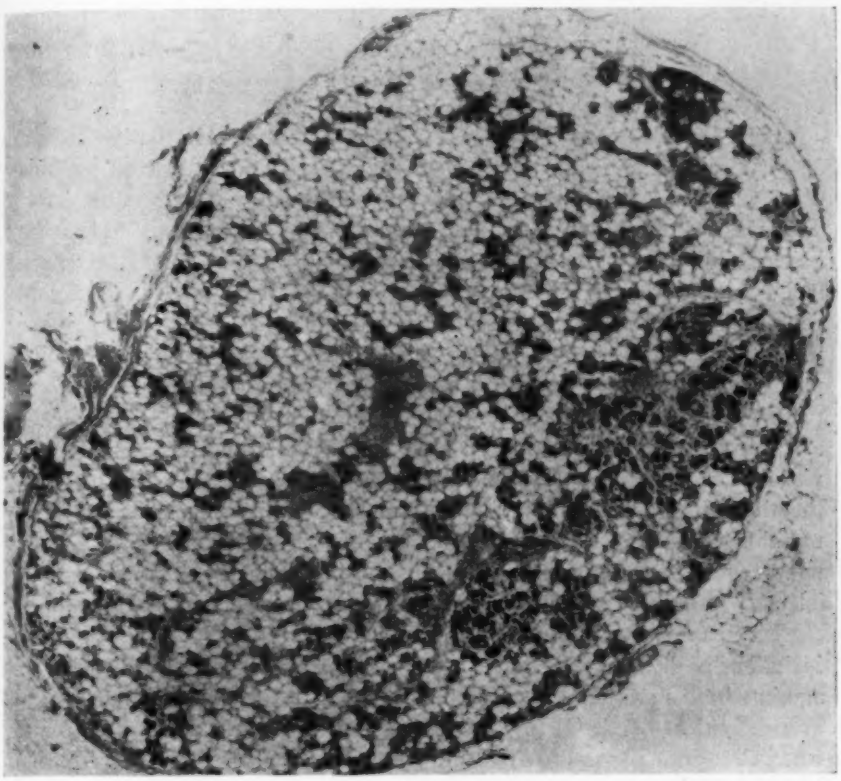

Fig. 3.—A cross section of a whole parathyroid, showing marked fatty changes as evidence of extreme atrophy.

previous reviews of cases of Simmonds' disease, but if cachexia is the sine qua non of this syndrome they should have been omitted.

Sainton and Rathery [3] reported the case of a 32 year old syphilitic woman who was believed clinically to have myxedema. She had no axillary or pubic hair and complained of headaches but had not lost

3. Sainton, P., and Rathery, F.: Bull. et mém. Soc. méd. d. hôp. de Paris **25**:647, 1908.

weight. Autopsy showed a sclerotic thyroid, which weighed only 12 Gm. There was also atrophy of the adrenals and genitals. The pituitary was replaced by a "mandarin"-sized cyst.

Fahr [4] reported the case of a 50 year old woman who had a spontaneous menopause following pneumonia at the age of 24. She had never been well since then and died after a three weeks' stay in a hospital, which she had entered because of abdominal symptoms. The clinical diagnosis was Addison's disease, based on the brown pigmentation of the skin. Autopsy showed atrophy of the adrenal cortex, the weight of the combined adrenals being only 3 Gm. There was no cachexia. The pituitary, although normal grossly, showed microscopically fibrosis of the anterior lobe.

Lindemann's [5] case is almost identical with ours. His patient was a 34 year old woman who married at the age of 20, contracted syphilis soon afterward and had a spontaneous menopause three years later. This was followed by a falling-out of axillary and pubic hair and of the hair of the eyebrows. In the year prior to examination she had dyspnea and asthma. Physical examination showed a woman who was small and pale but well nourished. The genitalia were infantile. Her temperature was elevated, and she died of bronchopneumonia after having become psychotic. Autopsy showed a sunken sella turcica, in which a completely atrophic pituitary was enclosed. Microscopically, the cells in the anterior lobe were markedly atrophic. The posterior lobe was not well visualized. The thyroid weighed 6.6 Gm. and was fibrotic. Two out of the three parathyroids found were small and atrophic. The adrenals together weighed 2.8 Gm. and showed atrophy but no fibrosis. The pancreas showed generalized atrophy of the chief cells but not of the islets of Langerhans. The endometrium and ovaries were also atrophic.

Jakob [6] reported 2 cases. One was a true case of Simmonds' disease, including cachexia. The other case was that of a woman 45 years of age in whom since her last pregnancy, ten years previously, symptoms had gradually developed. Her skin was dry, and the pubic and axillary hair was falling out. She died of tuberculous meningitis. Autopsy showed her to be fairly well nourished. The pituitary weighed 0.585 Gm., and the anterior lobe was fibrotic. The thyroid was normal in size but showed fibrosis. The adrenals weighed 6 Gm. The uterus was small, and the ovaries were atrophic. The abdominal organs showed microsplanchia.

Muller's [7] case was that of a 59 year old woman who died suddenly of apoplexy. There were no symptoms. Autopsy showed dry skin,

4. Fahr, T.: Deutsche med. Wchnschr. **44**:206, 1918.
5. Lindemann, E.: Virchows Arch. f. path. Anat. **240**:11, 1923.
6. Jakob, A.: Virchows Arch. f. path. Anat. **246**:151, 1923.
7. Muller, E.: Klin. Wchnschr. **2**:1576, 1923.

sparse axillary and pubic hair, a bloated appearance and an increased amount of subcutaneous fat. The pituitary weighed 0.13 Gm., and the anterior lobe contained only a few cells. The thyroid measured 4 by 2 by 1.8 cm. and contained very small follicles. No other observations were given.

Hirsch and Berberich [8] reported the case of a 57 year old woman with syphilis who at 40 lost her libido, at 52 had her menopause and at 53 lost the hair from her eyebrows and the axillary and pubic hair. She began to experience slow, difficult speech, weakness, fainting spells and chilly sensations. There was no emaciation, but there was some brown pigmentation of the face. The basal metabolic rate was — 17 per cent. Terminally a depressive psychosis developed. At autopsy the pituitary weighed 0.6 Gm., and microscopically the anterior lobe was composed predominantly of cholesterol crystals and hemosiderin. In one corner there was an area of normal anterior lobe. The thyroid was fibrous and atrophic and weighed 11 Gm. The adrenals were normal and the ovaries small and atrophic.

Falta [9] described a syndrome which he called *die multiple Blutdrüsensklerose* and reported 2 clinical cases without pathologic corroboration.

COMMENT

Given the microscopic observations alone in this case, one would have to call it a case of Simmonds' disease, but the absence of cachexia raises the question whether the small group of cases in which cachexia is not present should be singled out from the rest. Without cachexia a clinical diagnosis of Simmonds' disease is usually not considered, and for that reason alone this syndrome of pluriglandular insufficiency should be considered in the differential diagnosis in cases that to all intents and purposes are instances of myxedema, Addison's disease or some other endocrine insufficiency. Falta's 2 cases might well fit into this group, in which theoretically the patients should be treated with extracts of the anterior lobe of the pituitary. Falta, however, had no right to assume in his cases that all the endocrine glands would show sclerosis. Atrophy alone, without sclerosis, might well account for the insufficiency.

This distinction between sclerosis and atrophy brings up the subject of the causes and primary origin of the disease. If one assumes, as Falta did, that all the endocrine glands are sclerotic, this would be strong evidence that the disease did not begin in one gland but that the same injurious agent affected all the endocrine glands. On the other hand, if some of the endocrine glands are sclerotic and others only

8. Hirsch, S., and Berberich, J.: Klin. Wchnschr. **3**:483, 1924.

9. Falta, W.: Die Erkrankungen der Blutdrüsen, Berlin, Julius Springer, 1913, pp. 363-373.

atrophied, one might assume a primary lesion in one of the sclerotic glands and secondary atrophy of the others. It is only reasonable to suppose that the same injurious agent that produced sclerosis in one gland would affect the other glands in the same way. We have not been able to find record of any case in which all the endocrine glands showed sclerosis, and since in Falta's cases there were no autopsies there is no evidence to support the concept of a disease entity characterized by sclerosis of all the endocrine glands. In our case and in some of the others reported the only glands that showed sclerosis were the pituitary and possibly the thyroid. The parathyroids, adrenals and ovaries were only atrophic and showed no evidence of the cellular infiltration and fibrosis that one ordinarily sees following an injurious agent. We are forced to propose, therefore, that the disease originated in either the thyroid or the pituitary or in both and that the other glands were affected secondarily.

If we could show still further that either the thyroid or the pituitary was not primarily sclerotic but involved in fibrosis secondary to atrophy, our problem as to the original focus of the disease would be solved. Regarding the pituitary, there can be no doubt that the destruction of most of the cells of the anterior lobe, the fibrosis and especially the lymphocytic infiltration point to the previous presence of an injurious agent, infectious or chemical. The cellular reaction is the important factor. The lesion in the thyroid, however, is different. Here there is no cellular reaction. It is well known that normally the thyroid contains a goodly amount of fibrous stroma. When atrophy of the thyroid occurs, it of course concerns the epithelial elements; the more severe the atrophy, the more prominent is the fibrous stroma. When complete atrophy with focal disappearance of follicles occurs, the fibrous stroma assumes greater prominence and strongly resembles postinfectious sclerosis. In his monograph on the thyroid Wegelin [10] went so far as to say that often no sharp line can be drawn between atrophy and sclerosis. Although it was not definitely proved that the thyroid in our case was atrophied, the burden of proof rests with those who say that it was not. We feel, therefore, that the primary disease in our case was in the pituitary and that all the other endocrine glands were atrophied secondarily. The same reasoning probably applies to the cases in the literature, but without having seen the microscopic sections we can form no definite opinion.

Experimental evidence of the primacy of the pituitary in the clinicopathologic syndrome which we have described here has several angles.

10. Wegelin, C.: Schildrüse, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1926, vol. 8, p. 86.

When the thyroid gland is the primary site of destruction, as in spontaneous myxedema and cretinism, the anterior lobe of the pituitary is often hypertrophied (Rogowitch [11]). If the thyroid is removed in animals, a similar enlargement of the glandular portion of the pituitary occurs (Rogowitch). That this increase in the pituitary functional activity is more than a histologic one was shown by Hertz and Oastler,[12] who found increases in the thyrotropic hormone of the blood and urine of patients with myxedema. In sharp contrast to this, the assay on the urine of our patient as performed by the method of Hertz and Oastler was negative for thyrotropic hormone. The method of Hertz and Oastler makes use of hypophysectomized animals as test objects in a replacement type of experiment.

In rats, following hypophysectomy the thyroid, parathyroid, ovary and adrenal undergo atrophy. This is in complete harmony with the primary lesion in the pituitary, the secondary atrophy of the remainder of the endocrine system and the development of incomplete myxedema, hypofunction of the adrenal cortex and cessation of ovarian function (amenorrhea) in our patient. Hypophysectomized animals live for many months after operation, and following the atrophy of their endocrine glands they enjoy comparative health for many weeks, although growth ceases. Their metabolism drops; they become inactive, acyclic and hypoglycemic, and die in a condition of marked asthenia and stupor but in good nutrition. Hence, the analogy between the clinical course of our patient and the clinical course of animals suffering from pituitary insufficiency is striking. Considering the span of life of the rat as compared with that of our patient, the removal of the pituitary in a rat can be regarded as providing an approximately equal chronicity of pituitary insufficiency as that which must have existed in our patient, sufficient amounts of the hormones of the anterior lobe of the pituitary having been already secreted to "carry on" in the rat for a time after hypophysectomy.

Clinically the syndrome which we have described is to be distinguished from congenital pituitary aplasia, since growth, menstruation and pregnancy ensued in this patient in a normal fashion. The miscarriage which preceded the onset of amenorrhea may be interpreted as a part of the insidious onset of chronic pituitary failure, or it may have stood in some etiologic relation to the "secretory exhaustion" of the pituitary which is often suspected in true Simmonds' cachexia.

The absence of cachexia in our case was striking both during life and at the time of postmortem examination and deserves special mention. The patient was moderately obese. The obesity had no special distribu-

11. Rogowitch, N.: Beitr. z. path. Anat. u. allg. Path. **4**:453, 1888-1889.

12. Hertz, S., and Oastler, E. G.: Endocrinology **20**:520, 1936.

tion and was fairly generalized. The preservation of such excellent nutrition despite chronic failing health over a period of ten years is in sharp contrast with the rapid wasting which occcurs in Simmonds' cachexia following acute damage to the anterior lobe of the pituitary (infarct, hemorrhage or syphilis). Anorexia was prominent, and her restriction of her diet to carbohydrates is of considerable interest in view of what is known of the role of the pituitary in fat and carbohydrate metabolism. Chronic hypoglycemia may well have been the stimulus for such a change in appetite and may in part have explained her excellent nutrition at death. The aches in the bones and joints of which she complained so prominently may have been on a hypothyroid basis. No disturbance in bone metabolism was found which was sufficient to explain the aching. The high blood cholesterol, the characteristic findings in the electrocardiogram and the clinical appearance of the patient confirmed the diagnosis of myxedema and fitted with a basal metabolic rate of —28 per cent.

The results of the attempted thyroid therapy are worthy of special discussion. Dr. John H. Talbott and one of us (S. H.) have been struck by the sensitivity of patients with adrenal insufficiency to thyroid extract. In two definite instances we have reliable evidence of a precipitation of crises of adrenal insufficiency, which may be accompanied by nausea, vomiting, a high temperature, psychosis, coma and convulsive manifestations without a definite lesion of the brain. It is therefore quite likely that our patient died of adrenal insufficiency after the attempt to relieve her thyroid deficiency. Chemical evidence of this would have been available in determinations of the blood sodium if these determinations had not been vitiated by the administration of saline solution. The diagnosis of adrenal insufficiency could not otherwise have been made during life, although there was slight malar pigmentation.

It is important to recognize that this interesting possibility of chronic pituitary insufficiency may exist. Future research may give adequate pituitary fractions for complete replacement therapy. Such a condition should be suspected in all cases of typical myxedema or in cases of myxedema in which earmarks of other endocrine deficiencies are present, such as onset of early amenorrhea (myxedema is usually characterized by metrorrhagia) or signs or symptoms of adrenal insufficiency, and finally a negative thyrotropic assay of blood or urine in the untreated state of myxedema should point to a primary deficiency of the pituitary.

SUMMARY

A case of pluriglandular insufficiency without cachexia, clinically believed to be a case of myxedema, is described. Except for the absence

of cachexia, all the postmortem observations (fibrosis of the anterior lobe of the pituitary and atrophy of the thyroid, parathyroid, adrenal, ovary and uterus) are characteristic of Simmonds' disease. Pathologic and experimental evidence is presented to establish the primacy of the anterior lobe of the pituitary in the pathogenesis of the disease. The importance of recognizing this condition clinically and differentiating it from myxedema or any other primary endocrine insufficiency is emphasized. Only 6 other similar cases were found recorded in the literature, the most typical being that reported by Lindemann.

WALKER 256 RAT MAMMARY CARCINOMA IN VITRO

FURTHER OBSERVATIONS

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During the last several years a number of studies have been carried on in this laboratory relating to the strain of rat mammary carcinoma known as Walker 256. Earlier studies of this tumor in vivo and in vitro have been given in previous publications.[1] A group of later observations on this tumor in vitro are described in this paper.

MATERIALS AND METHODS

This strain was carried in D3.5 Carrel flasks. The solid culture medium used since first explantation of the cultures consisted of 1 part of chicken plasma without any anticoagulant added and 2 parts of a fluid culture medium composed of 40 per cent horse serum, 20 per cent extract of chick embryo and 40 per cent saline solution, of the formula previously described.[2] For the first year 1.5 cc. of this mixture was used in each culture flask. Later the total amount of solid culture medium used was 0.8 cc.

The relative percentages of the constituents were maintained unaltered, however. To each flask after clotting was added 1 cc. of the aforementioned fluid medium. This fluid medium was changed three times weekly. At each change the culture was rinsed with saline solution, and fresh fluid medium was added. The culture medium was adjusted to about p_H 7.5 by means of carbon dioxide.

In general from one to four explants were used in each Carrel D3.5 flask. More recently strip cultures, previously described,[3] were used. From one to three strips were placed in each flask.

The tissue from which the cell strain used in this work was taken was explanted from the (approximately) one hundred sixty-third generation of subinoculation of Walker 256 in rats. This was explanted into tissue culture April 10, 1936. This strain of cells will be identified as strain X169 to distinguish it from the strain previously described, identified as strain X143. To date strain X169 has grown twenty-two months in vitro.

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1. Earle, W. R.: Am. J. Cancer **24**:566, 1935. Earle, W. R., and Voegtlin, C.: IV. A Comparison of the Growth of the Jensen Rat Sarcoma in Subcutaneous and Intramuscular Transplants, in Experimental Studies on Cancer, National Institute of Health Bulletin 164, United States Treasury Department, Public Health Service, January 1935, pp. 47-58. Earle, W. R.: Arch. f. exper. Zellforsch. **20**:141, 1937.

2. Earle, W. R.: Arch. f. exper. Zellforsch. **16**:116, 1934.

3. Earle, W. R.: Arch. Path., this issue, p. 88.

RESULTS

After explantation, the early history of this tissue strain was essentially similar to that of strain X143. In the early generations there were many fibroblasts present, but at about one hundred and twenty-five to one hundred and seventy-five days after explantation these had almost or entirely disappeared, and there was left in the culture a cell type which seemed to coincide with the epithelial cell previously described as series X143.

Repeated examinations of the strain during the last year both in living and in fixed and stained preparations have shown no cells other than what appeared to be the epithelial cells of the tumor.

The rate of increase in the diameter of the culture was quite rapid and coincided closely with that previously observed for strain X143. From about one hundred and seventy-five days on, i. e., after elimination of the fibroblasts from the culture, growth was uniform. So long as 1.5 cc. of plasma was used on the cultures, it was found that the best results were obtained by transferring the cultures to fresh flasks about every ten to fourteen days, since if they were allowed to run longer central necrosis set in. This central necrosis did not seem to interfere with the peripheral growth of the culture, however, and in cultures allowed to run longer than this time it was quite the usual thing to see extensive central necrosis, with numerous mitoses in the peripheral areas of the cultures. Such cultures frequently increased to a diameter of about 18 mm. before the necrosis started reaching out into the peripheral zones of the culture.

In instances in which the cultures showed marked central necrosis, it was found that in transfer this central necrotic zone often tended to dissect away from the living culture in a single mass, thus leaving a fringe of healthy living cells which could be easily transferred and which would grow vigorously. In transferring these fringes it was soon found that excellent growth could be obtained from relatively small clumps of cells, in fact far smaller clumps than it has been possible to use for routine transfers of fibroblasts.

During the later history of the strain the amount of solid clot in each culture was reduced, as stated, from 1.5 to 0.8 cc. This gave a thinner clot but one not too thin to handle with facility. In this thinner clot the cultures required explantation less frequently. A transfer period of about twenty to thirty days was satisfactory, and even with this extended time there has been little or no marked central necrosis such as was seen with the thicker clot. With the thinner clot, cultures were carried up to about sixty days or in some instances even longer without massive central necrosis. This was particularly true with any culture which was started with a small fragment of the thin fringe of cells left after the necrotic center of an old culture had been discarded.

In some of the later work an attempt was made to use strip explants as described in a previous article.[3] For raising relatively large quantities of this tissue fairly rapidly, this technic yielded such satisfactory results that finally no other type of culture was used. From one to four strips were used in each Carrel flask. Probably two strips gave the most satisfactory results. Growth was often a little ragged for the first few days, but after that it was generally quite uniform along the length of the strip. In a flask containing three strips almost the whole floor of the flask was covered with the living tissue within a relatively short time. For explantation the strips were each split lengthwise to give strips not more than from 1 to 2 mm. wide and as long as desired. A convenient length was from 10 to 15 mm. Longer strips showed a tendency to curl laterally.

Strip cultures taken from thin clots such as those described were the most satisfactory. Cultures grown in these thin clots, and some grown in even thinner clots for long times, i. e., in excess of sixty days, often showed so much liquefaction that it interfered to some extent with their symmetry. In the few instances that liquefaction really interfered with the growth or with the examination of the culture it was easily controlled through patching the clot by a single addition of fresh plasma, horse serum and embryo extract and draining off any surplus added after the culture had been flooded with the mixture.

Another phenomenon which was almost never seen in cultures in the thick clots but which was observed a number of times in cultures in the thin clots was the appearance of small subcultures within the flask at some distance from the original clump. In all instances in which this was noted, it occurred in relatively old cultures, in which the slight liquefaction probably served to cut loose small clumps of cells or isolated cells from the original explant. Cultures were seen in which clumps of as few as three or four cells seemed to be living normally and vigorously (fig. 1), while subclumps of graded large and rapidly increasing sizes indicated that such clumps were not only surviving but growing rapidly.

With the introduction of the thinner clot and the consequent prolongation of the life of the culture, the morphologic changes in the culture were far more accentuated and hence seemed far more remarkable than similar changes observed in the culture in the thicker clot. Following explantation of the new culture the first cells to migrate out into the surrounding clot were elongated spindle-shaped or flattened spindle-shaped cells (fig. 2*A*). This continued until at about nine days the culture consisted more or less of a sheet of flattened spindle-shaped cells of the type shown in figure 2*B*. There was a tendency for the sheet of cells to form at the glass-plasma or the fluid-plasma interface of the clot as with most other types of cultures. At the same time that this dense growth of spindle-shaped cells was seen coming out and forming

sheets on the interfaces of the plasma, a far sparser growth generally occurred and progressed within the clot itself. This growth showed a tendency to form long interlacing branching strands of cells as shown in figure 3.

As growth continued, in addition to an increase in the area of the culture there was a thickening of the central part of the culture. From

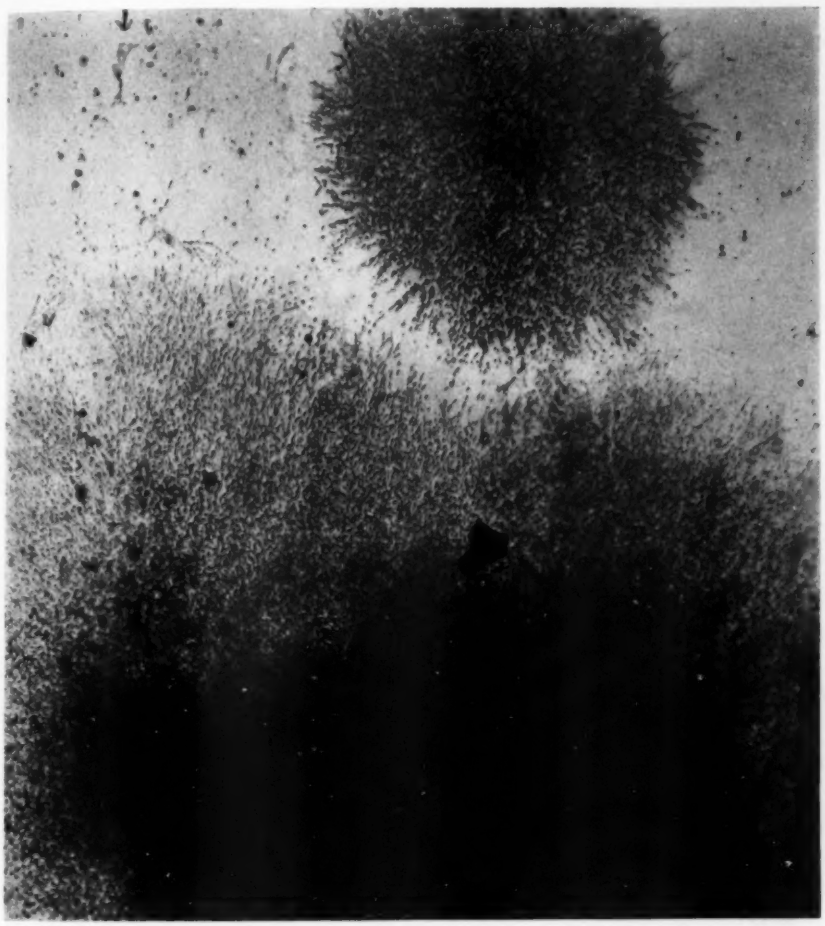

Fig. 1.—A low power view of a culture of the cell strain; × 10. This culture had been carried in the same flask and without any patching of the clot for about forty-five days. Note the clump of cells which has transplanted itself and which has already reached a relatively large size. Note also in the upper left hand corner even smaller independent cell clumps. This photograph and all subsequent photographs were made of the cell strain after it had been carried in culture about a year and a half.

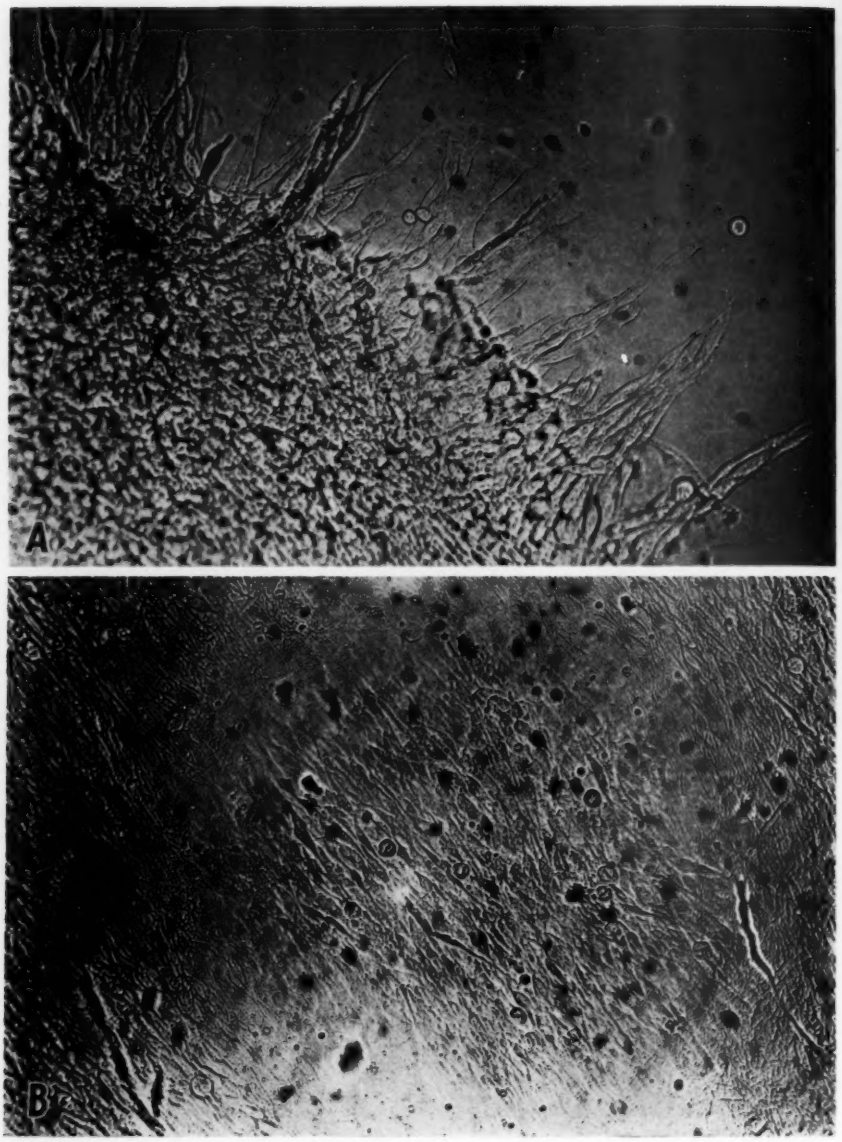

Fig. 2.—*A*, two day old culture of the tumor; × 133. Note the generally elongate shape of the cells in the fringe migrating into the plasma. *B*, area in a culture approximately forty-five days old; × 133. The field chosen is near the edge of the culture. Note that the cells are in general of spindle shape and that they are so close together as to form an almost solid sheet. This type of growth is also typical of the more central areas of younger cultures.

a thickness of just a few cells this region became many cells thick. In contrast with a luxuriantly growing fibroblast culture, this region of the Walker 256 culture became so much thicker that without any lens at all the two cultures could easily be differentiated at a glance. Apparently as a result of the thickening of this central area, with consequent crowding, the cells of this region showed a tendency to lose their spindle form and to become increasingly rounded. Later, the crowding became so severe that the cells were squeezed into definitely polygonal shapes. This process of rounding and forcing into polygonal shape finally

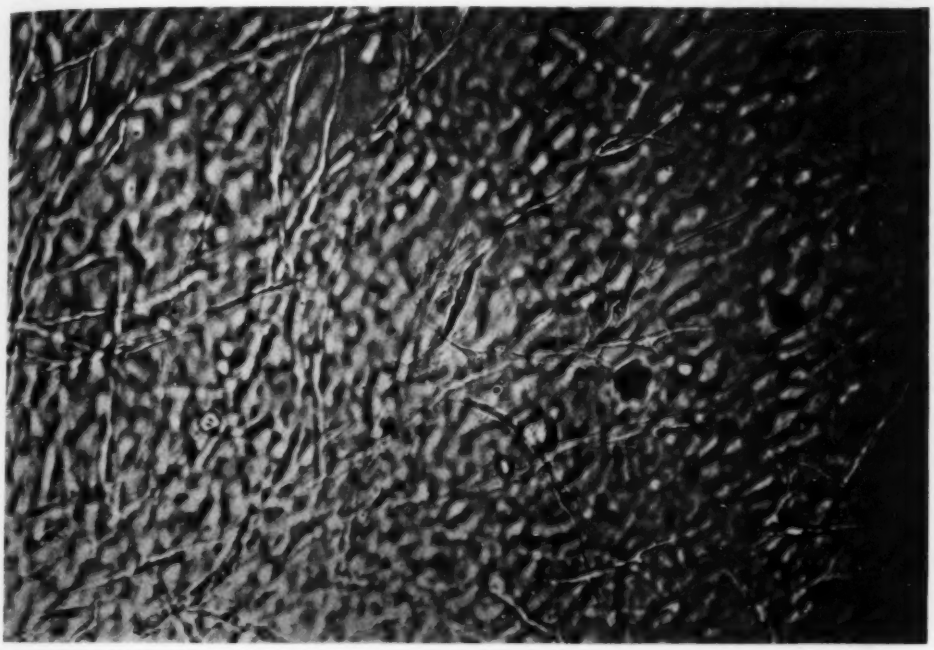

Fig. 3.—A culture about forty-five days old, focused to show the migration of cells as loose fibroblast-like strands within the clot itself; × 154.

reached the extreme condition shown in figure 4. The area shown in this photograph is in no way exceptional; it is entirely typical of the thickened central regions that were observed in such cultures. At a later stage the closely crowded central area began to show massive necrosis. This massive central necrosis, as is obvious from the extreme crowding of the cells, was probably due entirely to this crowding.

The same steps in the growth of a culture were also noted with equal clarity in passing from the center to the periphery of one of these old cultures.

If an explant was cut out from the densely crowded polygonal cell areas of such an old culture before necrosis set in, and if this explant

was transferred to fresh clot, the morphologic cycle started over, and the first migrating cells were the elongated spindle-shaped cells seen in figure 2 *A*, which, incidentally, was taken from just such an area.

The number of mitoses in rapidly growing cultures of this tumor cell was often very great. Low power microscopic fields were seen with as many as 23 cells in cleavage. In the negative of the photomicrograph reproduced in figure 2*B*, for instance, which is larger and sharper than the reproduction made therefrom, 12 cells in mitosis were counted. Other fields with up to 23 cells in mitosis have been seen.

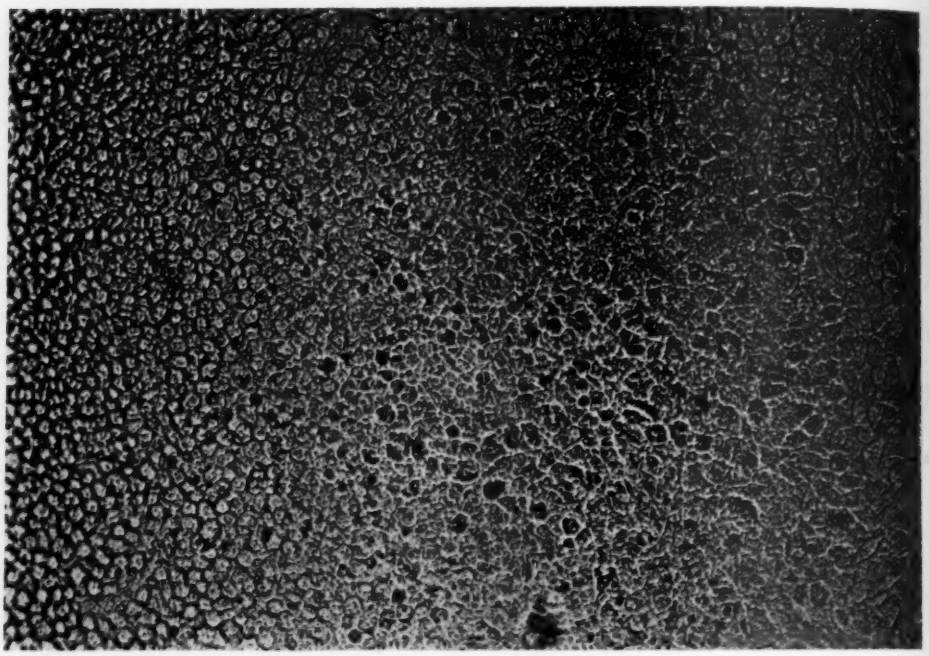

Fig. 4.—An area from the same culture as that shown in figure 3 but rather close to the center of the culture; × 154. The left end of the photograph is toward the center of the culture, being about 3 mm. from the center.

COMMENT

At no time in its life has this strain of cells received extracts of rat tissue or rat serum. There seems to be no question that the strain can not only grow in the complete absence of such solutions but continue to grow luxuriantly. This is in strong contrast with the cells of the normal mammary gland, from which the tumor arose, and which we have repeatedly attempted to grow in this and other culture mediums, without the least success.

With increasing age the cyclic changes in the morphologic character of these cultures represent alterations in the morphologic character of one strain of tumor epithelial cells under different conditions of crowding of the cells in the culture. While a diversity of cell form has been noted in tissue cultures for many types of cells cultivated, the cycle of change just described seems a really remarkable instance of the great variety of morphologic changes which a cell strain can show in culture.

In particular, it is desired to emphasize that while one is accustomed to think of epithelial cells, including malignant ones, as showing rather characteristically lobulated sheets in tissue culture, the changes in this strain of Walker 256 tumor epithelium demonstrate that at least this epithelium from the malignant mammary gland can not only show characteristically lobulated sheets of tissue but also and more frequently fibroblast-like interlacing strands, loose cells, sheets of elongated spindle-shaped and flattened spindle-shaped cells and dense sheets or masses of rounded or polygonal cells, the masses often many cells thick. In the identification and classification of epithelial cells, particularly malignant epithelial cells, this remarkable potentiality of varied morphologic change in the architecture of the culture must be carefully considered.

As noted, the architecture of the normal fibroblast culture grown under identical conditions is quite different from that of the cultures just described. In the normal fibroblast cultures, even in older cultures, I have never seen the extreme cell crowding near the center of the culture which was seen with the epithelial cultures described. From my observations it is believed that an essential morphologic difference between the two types of cultures has lain in the fact that while the rate of cell multiplication in the fibroblast culture is rapid the cells have a strong tendency to migrate out and away from the center of the culture. With the epithelial culture, however, although the diameter of the culture has generally been consistently less than that of the fibroblast culture, the number of cells seen in mitosis is far greater than has been observed in the fibroblast culture. It has appeared that this condition of rapid proliferation and relatively more limited migration has resulted in a far more densely crowded culture than is seen with the fibroblast.

SUMMARY

At the present writing the epithelium of the Walker rat mammary carcinoma has been grown in apparently pure culture for twenty-two months. During this time no extractives from rats have been used, the culture fluid having consisted of horse serum, extract of chick embryo and saline solution. In cultures grown from strip explants growth has been luxuriant and particularly satisfactory. The range of cell shape has varied greatly and has included types of cell shape not generally associated with epithelial cells.

USE OF STRIP-SHAPED EXPLANTS IN TISSUE CULTURES

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During the course of some previous work with large and very dense tissue cultures, the cells of which were so clear that they could be seen only with great difficulty,[1] the necessity arose for obtaining some idea of the cell density throughout the extent of the culture. To accomplish this a slender strip was cut out from the culture. The length of the strip was the diameter of the culture; the thickness was that of the culture contained in its plasma clot, and the width of the strip was about 2 mm. This strip was explanted into fresh culture medium. The growth of this strip was so luxuriant that the question was raised whether a strip form of explant instead of the usual almost square or slightly elongate form does not offer marked advantages for much or most tissue culture work.

MATERIALS, METHODS AND RESULTS

All cultures were grown in Carrel D3.5 flasks. The solid culture medium used consisted of 1 part of chicken plasma, without anticoagulant, and 2 parts of a mixture of 40 per cent horse serum, filtered under pressure, 20 per cent chick embryo extract and 40 per cent saline solution of the formula already given.[2] In the first part of this work a total clot volume of 1.5 cc. was used; with later cultures the volume was 0.8 cc., while for some few cultures it was even lower, e. g., about 0.3 cc. In all cultures 1 cc. of fluid culture medium of the composition set forth was added to each culture. The fluid was changed three times each week, and at each change the cultures were first rinsed with saline solution of the formula given.[2] The hydrogen ion concentration of all solutions was adjusted to about p_H 7.5 by means of carbon dioxide.

Five different types of tissue were used in this work. The first type was rat subcutaneous connective tissue. The second was sarcoma which arose in the subcutaneous tissue of a rat following an injection of 10 mg. of methylcholanthrene about two hundred and twenty-five

1. Earle, W. R.: Am. J. Cancer **24**:566, 1935.
2. Earle, W. R.: Arch. f. exper. Zellforsch. **16**:116, 1934.

days previously. The third was the Walker 256 rat mammary carcinoma, strain X169, and the fourth and fifth were freshly explanted mouse subcutaneous tissue and rabbit tunica vaginalis. In the instance of the sarcoma strip, cultures were studied from first explantation through about one hundred days of growth. For the carcinoma, strip cultures were studied at intervals from about nine months to about twenty-two months after the original explantation. The rat subcutaneous connective tissue was studied through about one year of growth, while the rabbit tunica vaginalis and mouse subcutaneous connective tissue were carried for only short periods.

All handling in the explantation and transplantation of the cultures was done with the usual instruments that were used as a routine. These consisted of a platinum-iridium wire flattened on the end and mounted in a glass handle, and two iris knives.

The explants used were of many sizes. In taking explants from fresh solid tissues, such as the tumor tissues, best results were obtained by cutting thin slices—as thin as was practicable freehand. After the first few trials an attempt was made to hold these slices to a thickness of about 0.5 mm., as such slices were the thinnest that could be cut conveniently. The areas of the slices were limited only by the size of the tissue fragments available. From these slices strips were cut. Of the different sizes tried, strip widths of from 1 to 2 mm. gave the most satisfactory results, and in later work with dense tissues an attempt was made to cut all tissues about 1.5 mm. wide. Wider explants of this thickness or thicker ones showed some central necrosis.

In instances of a loose tissue, such as adult rat or mouse subcutaneous connective tissue, which incidentally has the extremely troublesome faculty of adhering tenaciously to any slight irregularity on the dissecting instruments, explants up to 3 mm. in width were used without marked complications. While cuts were made as clean as possible, it was recognized that with these loose tissues there occurred much more pulling and tearing of the tissue than with the more solid tissues.

While it was found that the length of the explant could be varied within extremely wide limits, for handling in D3.5 flasks a length of about 15 mm. and not more than 20 mm. was found most satisfactory. When this length was used, several explants could easily be arranged in a Carrel D3.5 flask before the clot solidified. When just a single explant was used in the flask, a longer explant could be used if there was some definite reason for doing so, but in general it was found that the explants about 25 to 30 mm. long had a marked tendency to curl laterally. While this curling rarely interfered with cell migration, it did make the later handling of the strips more difficult.

In a few instances in which cultures of rat subcutaneous connective tissue were grown in D80 flasks, it was found possible and practicable

to use as a routine strips up to approximately 50 mm. in length. Difficulties of handling did not warrant exceeding this length.

Frequently the size of the tissue fragment available was so limited that adequate strips for explantation could not be cut. In these instances fragments were cut as close to strip shape as possible and then arranged lengthwise, very close together, in rows in the culture flask. In a number of instances this method gave as good strip cultures as if the tissue had been cut out in strip form originally. On a few occasions, with cultures under less favorable experimental conditions, growth was not as good as was expected, and fusion of the cultures was so incomplete that the

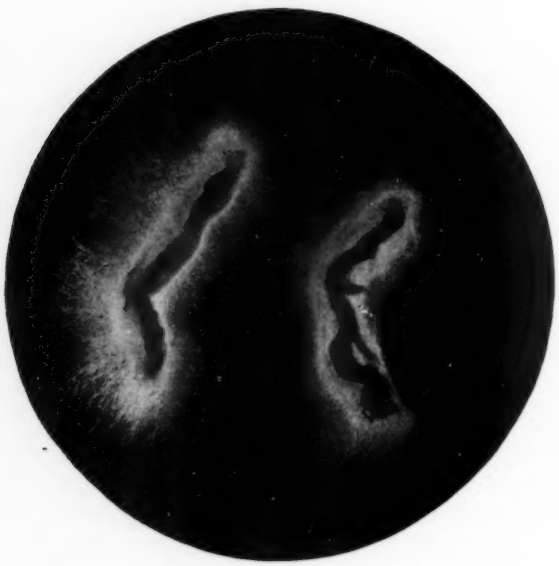

Fig. 1.—Seven day old strip cultures of a subcutaneous sarcoma caused by injection of methylcholanthrene in a rat; × 3. This tissue had at this time been carried through five generations of transplants in vitro.

strips obtained were not regular in their growth. Because of this observed irregularity, the attempt was always made to cut the original strips for explantation as close to the desired length as possible, and particular care was taken with cultures known to show slow growth or to be under toxic conditions.

In starting strip cultures from cultures already growing, if the cultures were adequately large and free from necrosis, strips were satisfactorily cut out directly from the culture. If the culture size was inadequate, the strips could generally be "synthesized" satisfactorily from a number of smaller cultures as described for fresh tissues.

In the handling of all strip cultures the clot thickness given by 0.8 cc. of clot was found preferable to that given by 1.5 cc., since with the thicker clot the explant strips had a much greater tendency to turn over on edge than with the thin clot, while central necrosis was much more

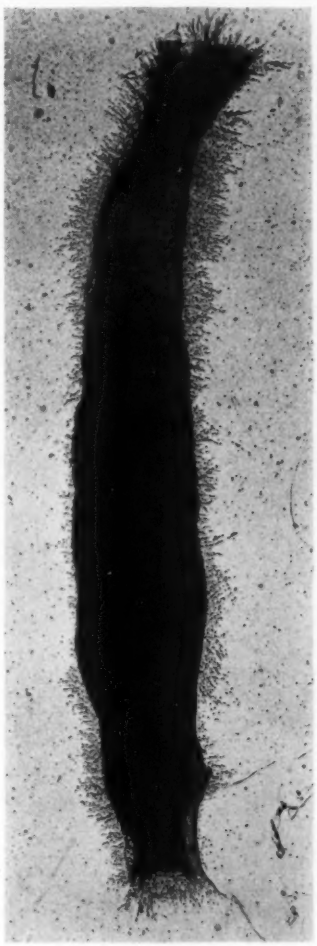

Fig. 2.—A two day old strip culture of the epithelium of a Walker 256 rat mammary carcinoma; × 8. Note the fairly even migration of the cells. This culture and the one shown in figure 3 were from a strain of cells which had been carried a year and a half in vitro.

prominent and started earlier. For these reasons in the later work the thinner clot was used as a routine.

In the explantation of strip cultures it was found that they could be dissected out, cut to shape and explanted in appreciably shorter time

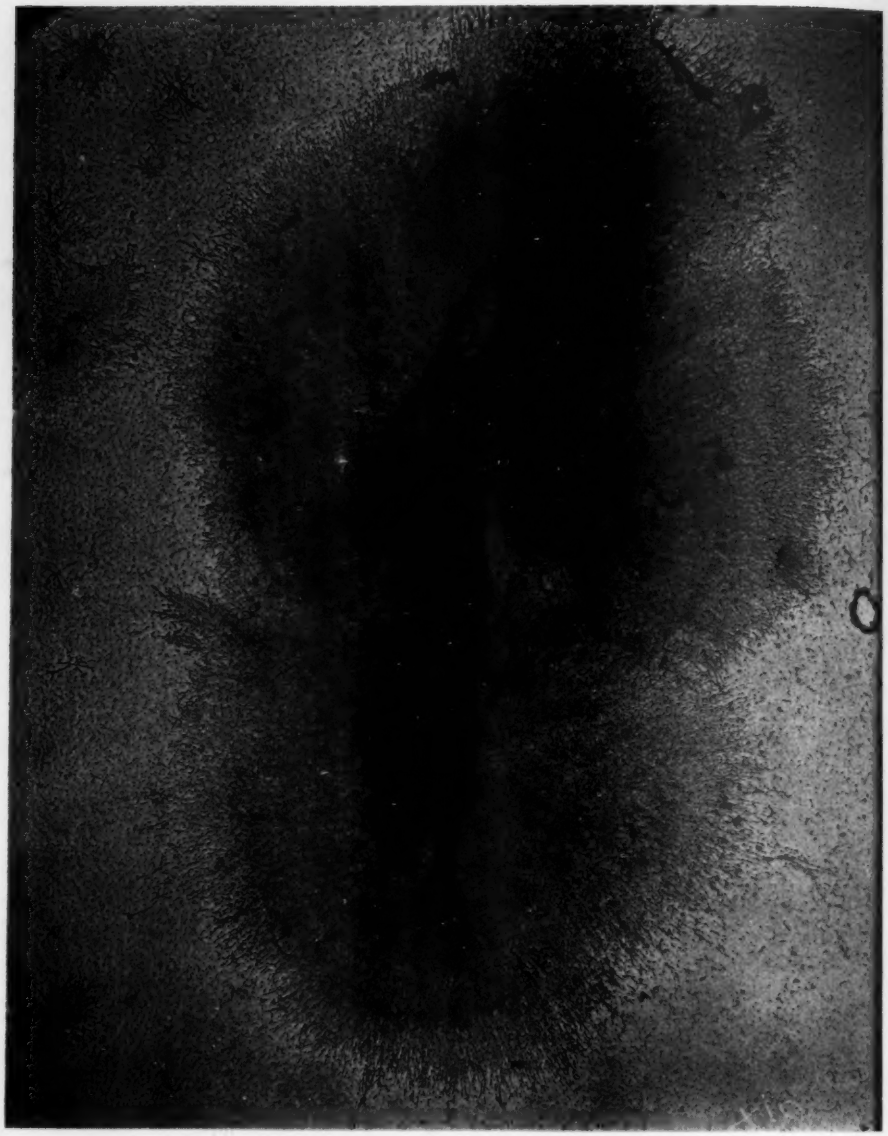

Fig. 3.—Forty day old strip culture of the Walker 256 rat mammary carcinoma; × 6.6. Note the luxuriant even growth and freedom from massive central necrosis.

and with far greater ease than cultures of the usual shape in equivalent amounts. In this explantation, after the whole clot sheet had been slid out on a sterile glass plate and the edges of the culture trimmed in a manner identical with that used for the usual shapes of cultures, a cut was made down the midline of the length of the culture, separating it into two lateral halves. If the parent culture had been allowed to grow so long that it showed considerable central necrosis, this necrosis was sliced off. If necessary because of excessive length as a result of luxuriant growth, the two strips were then cut halfway of their length. The cultures were then placed in saline solution for replanting.

With rapidly growing tissues which were to be allowed to grow for some time, it was found unwise to explant more than two or at most three such 15 mm. strips in a D3.5 flask. For cultures which were to be carried as long as possible without explantation, one explant gave the best results.

The character of growth seen from strip cultures did not differ appreciably from that of growth from the more usual type of culture. If anything, under the conditions used, the initial migration possibly came out a trifle slower in some instances. Once started, the growth of the strip culture was quite as rapid as that of the usual culture and in many instances appeared to be even more rapid.

The time during which any strip culture remained free from central necrosis was comparable to the time during which a culture of the usual shape and a diameter equal to the width of the strip culture would remain free. Strip cultures of connective tissue have been observed growing rapidly and entirely free from any massive necrosis seventy-five days after explantation, while with cultures of methylcholanthrene sarcoma marked central necrosis appeared after ten days in both usual and strip cultures. It should be noted, however, that even with cultures of the latter type of cell, which regularly showed early central necrosis, peripheral growth continued at an extremely rapid rate far in excess of sixty days.

COMMENT

Under the conditions described, the strip type of explant has seemed particularly adapted for carrying stock strains of cultures and for making cultures when large amounts of tissue were required.

It should be understood that the strip culture is not in any sense recommended as an absolute substitute for the usual types of tissue culture explants. It is sufficient to say that with cultures of the cell strains studied for the last year the method has given results both in ease of handling of the cultures and in the growth of the cultures themselves such that nearly all the cultures in this laboratory have been or are being shifted over to the strip form.

The fact that growth can be obtained from extensive strips of tissue and that rapid and continued proliferation can be observed in such cultures, with quite large final sizes of the cultures, raises the question whether this finding may be extended even further, so that by only slightly modified and relatively simple methods tissues can be cultivated from explants in sheet form. Experience with the strip cultures indicates that the chief difficulty which arises is the question of the diffusion of oxygen, carbon dioxide, food and waste substances through the culture. If this concept is correct, it is obvious that if one keeps the thickness of the culture down so low as not to interfere excessively with this nutritional exchange, there would be little or no limitation on the size of explant which might be used. In other words, there seems to be little reason why one should not grow cultures from extremely thin slices or membranes of tissue of far larger surface area and tissue mass than any now in use. It is suggested that just as Warburg and his successors succeeded in keeping tissue slices several tenths of a millimeter thick alive long enough to follow the respiratory changes of the cells over many hours, there seems no obvious reason why similar slices should be not only kept alive but be made to live and grow in cultures for much longer periods of time under relatively simple cultural conditions and under conditions which allow to at least some extent microscopic examination in vitro. While I have not had a chance as yet to verify this by adequately extensive experiments, it may be noted that I have succeeded in culturing an area of 1 sq. cm. (approximate) of tunica vaginalis of the rabbit. This tissue was grown for in excess of thirty days and at the end of that time was still in a condition of healthy and vigorous growth.

It is suggested that if such thin tissue slices can be grown successfully this will allow raising in culture relatively much larger amounts of certain living tissues than can be grown easily by current methods. Further, with the culture of organ and tissue slices in vitro, another technic is available for the study of factors controlling cell differentiation, cell regeneration and tissue architecture within the organ slice.

SUMMARY

A method of growing tissue cultures from strip-shaped explants is detailed. The use of these strip-shaped explants has allowed the routine growth of cultures of large size from the five different types of tissue used. This type of culture has been found easy to handle. The extension of this method of culture to the growth of tissue from even larger, sheet-shaped explants is discussed, and an instance of such successful growth is given.

MALIGNANT TUMOR OF THE GREATER OMENTUM SIMULATING A GLOMANGIOMA

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AND
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The first authentic case of a glomus tumor was described by Kolaczek [1] in 1878 as an angiosarcoma. Such tumors had probably been known for a long time under other names, such as "perithelioma," "endothelioma," "neuroma," "myosarcoma" and "painful subcutaneous tubercle."

In 1920 Barré [2] reported the case of an 18 year old girl with such a tumor of the middle finger of the right hand. In 1922 he reported 3 more cases.[3]

It remained for Masson [4] to give a name to these peculiar tumors. Because of their similarity to the gland of Luschka (glomus coccygeum), he called them glomus tumors. Masson's first paper mentioned 3 cases, and in 1927 he and Gery [5] published a report of 4 more.

After this, many cases were reported, particularly in the foreign literature. The first notable contribution in English was that of Greig,[6] who reported 3 cases of his own and listed 23 from the English and Scotch literatures.

In this country Mason and Weil,[7] in 1934, were the first to report a case. Their report was followed by Adair's [8] report of 10 cases, a study of 11 cases by Stout,[9] who also reviewed the literature, a report of 17 cases by Lewis and Geschickter,[10] a report of 7 cases by Bailey,[11] and reports of other cases by various investigators, including Raisman

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From the Department of Pathology, Cook County Hospital, and the Department of Surgery, Northwestern University Medical School.

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and Mayer,[12] Burman and Gold,[13] Livingston,[14] Love,[15] Jirka and Scuderi,[16] Mackey and Lendrum,[17] Theis,[18] Radasch,[19] Slepyan[20] and Kolodny.[21]

Altogether, we have found reports of 106 cases.

ANATOMIC FEATURES

The normal glomus, from which the glomus tumor originates, is a direct arteriovenous anastomosis. Glomuses are widely distributed over the body. They occur most commonly in the skin of the palms of the hands and of the terminal phalanges of the fingers, within the bones of the terminal phalanges, in the nail beds, in the skin of the lips, nose and eyelids and at the tip of the tongue. They may also occur in the tendons, gums, pia mater, dura, iris, ciliary bodies, endocardium and elsewhere.[22]

Sucquet[23] called these structures *canaux dérivatifs*, and Hoyer[24] completed the description. Masson used the term "neuromyoarterial glomus." Popoff[25] made a thorough study of the digital glomus, which he described as consisting of an afferent artery, an arteriovenous anastomosis proper (the Sucquet-Hoyer canal), preglomic arterioles, a periglomic zone with a neuroreticular mechanism, collecting veins and an outer collagenous zone.

The function of the glomus has been well established by Grant and Bland[26] and Lewis and Pickering[27] as a means of controlling arteriovenous circulation in the digits and regulating both the local and the general temperature of the body.

Histologically, the media of the vessels is composed of epithelioid muscle cells which have unmyelinated nerve fibers in direct communication with their processes. These cells are called glomus cells.

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CLINICAL FEATURES

Glomus tumors are not common, although they are undoubtedly often diagnosed incorrectly.

They are about equally distributed between the sexes. They occur from early childhood to advanced old age but are perhaps commonest in the middle decades. About 40 per cent of the patients give a history of trauma.

The tumors usually occur on the extremities and are most frequently subungual in the fingers. They are usually described as being small and bluish and are nearly always associated with attacks of excruciating pain on contact.

They are sometimes accompanied by disturbances of the sympathetic nervous system.

They usually occur singly, but multiple tumors have recently been reported by Weidman and Wise [28] and by Bergstrand.[29]

Location of Glomus Tumors in One Hundred and Six Cases (Total Number of Tumors, One Hundred and Twenty-Five)

Upper Extremity (81)	Lower Extremity (31)	Elsewhere (13)
Hand (48): thumb (5), palmar surface of fingers (10), dorsal surface of fingers (2), palm (2), subungual tissues (29)	Foot (5): subungual tissues (2), elsewhere on foot (3)	Wall of chest (1)
Forearm and wrist (15)	Leg (6)	Coccygeal body (2)
Elbow (5)	Knee (7)	Widely distributed over skin (1)
Upper arm (6)	Thigh (11)	Location not given (9)
Acromial region (1)	Location not given (2)	
Location not given (6)		

In a thorough review of the literature we have been able to find records of only 2 cases in which a glomus tumor was malignant. The first case was that of a perithelioma of the coccygeal gland in an 11 month old child, reported by Hleb-Koszanska.[30] There were no metastases, but the author found histologic evidence of malignancy. The second case, reported by Kofler,[31] was that of a 50 year old woman in whom a tumor of the small pelvis was found incidentally during a gynecologic operation. Histologically this tumor showed spindle cells, palisading of nuclei and variability of nuclear structure. There were no metastases.

Concerning the tumor of the coccygeal gland of Luschka,[32] Reuther [33] suggested that it may be a factor in certain cases of coccygodynia.

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DISTRIBUTION OF LESIONS

As previously stated, glomus tumors may occur anywhere, although Freudenthal, Anderson and Weber [34] stated that these tumors in sites other than the hands and feet must be looked on as heterotopic formations.

The locations in which the glomus tumors in the 106 cases reviewed were found are given in the table.

The case we are reporting is unique in that (1) it is the first case reported of a metastasizing glomus-like tumor and (2) it is the first case reported, so far as we can find, of such a tumor involving the greater omentum and abdominal viscera.

REPORT OF A CASE

A Filipino aged 40 years was admitted to the Cook County Hospital, May 5, 1937, complaining of a mass in the abdomen which had been present for six months and not associated with any pain. He stated that five months prior to admission he noticed that his skin had turned yellow and that his stools were black. He admitted that he drank alcoholic beverages to excess and that he had had gonorrhea in 1919.

He was well developed and well nourished. The temperature was subnormal; the pulse rate, 72; the respiratory rate, 20. The blood pressure was 150 systolic and 100 diastolic. The scleras were icteric, and the heart and lungs were essentially normal. Palpation of the abdomen revealed a large mass which filled the entire right upper quadrant and extended down to the umbilicus. The mass seemed movable with the respirations, and the edge was smooth and sharp.

The hemoglobin content of the blood was 49 per cent; the red cell count, 2,750,000; the white cell count, 9,900, with 88 per cent polymorphonuclear leukocytes. The stools were tarry grossly and on chemical examination were positive for blood. The urine was normal. The icterus index was 6.25. The Kahn test of the blood was negative. On visualization with thorium dioxide the liver and spleen appeared within normal limits. In July 1937 the patient was transferred to a surgical ward for exploration of the abdomen.

At operation the stomach appeared enlarged because of what was thought to be an inoperable sarcoma. Two pieces were removed and examined by the late Dr. R. H. Jaffé. The first piece suggested a malignant neoplasm with origin in the nervous system, possibly a schwannoblastoma, while the second showed an angiomatous neoplasm.

The patient made an uneventful recovery and left the hospital. He returned two months later. This time he complained of weakness, shortness of breath, loss of weight and enlargement of the abdomen. He appeared anemic and weak. Rales were heard in the bases of both lungs. The liver extended 4 fingerbreadths down, and there was fluid in the abdomen.

The mass in the abdomen was slightly tender, and large lymph nodes were palpated in the inguinal region.

Roentgen examination of the bones and chest showed nothing abnormal. An abdominal paracentesis yielded 3,900 cc. of amber-colored fluid, which microscopically showed large islands of degenerated cells, the identity of which could not be determined.

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34. Freudenthal, W.; Anderson, R. G., and Weber, F. P.: Brit. J. Dermat. **49**:151, 1937.

The patient had much abdominal pain, which was relieved after tapping the abdominal cavity. He grew weaker and died in five weeks, approximately one year after the onset of his illness.

Necropsy.—The body was markedly emaciated and the skin a dirty gray-brown. The abdomen was distended and showed a scar in the midline and the wounds of two recent paracenteses. The abdominal cavity contained about 1,000

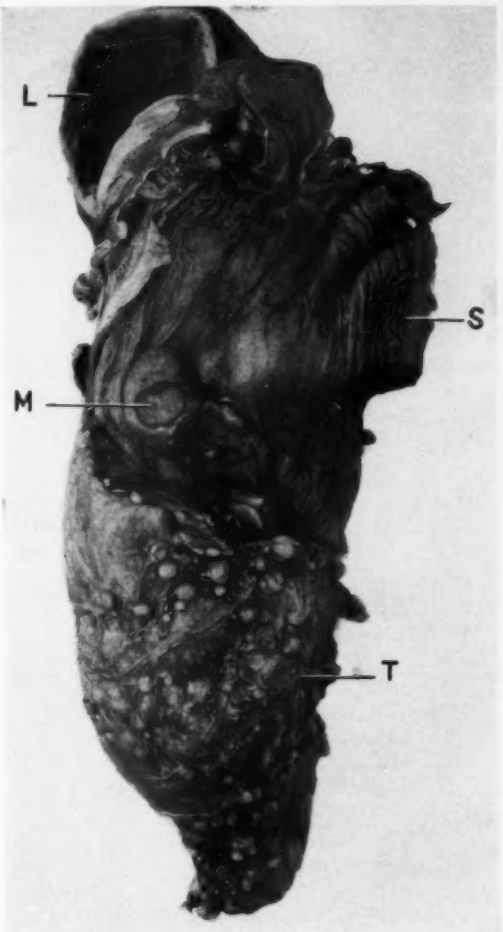

Fig. 1.—Tumor of the omentum (*T*) invading the stomach (*S*) with ulcer formation (*M*) and a single metastasis in the liver (*L*).

cc. of dark brown cloudy fluid. The greater omentum was transformed into a large firm mass which extended down into the pelvis (fig. 1). The surface was nodular, light purple-gray and glistening. The intestinal loops were displaced to the left by the mass, and in the mesentery as well as in the parietal peritoneum there were numerous nodules and plaques averaging 27 mm. in diameter. These were composed of a slightly transparent grayish tan or purple-gray tissue.

The omental mass measured 32 cm. in vertical diameter, 15 cm. in transverse diameter and 11 cm. in anteroposterior diameter. It could be separated with little difficulty from the small intestine, and it covered the anterior aspect of the hepatic flexure of the colon and the right half of the transverse colon. Below the left lobe of the liver there was a cavity, 8 cm. in diameter, filled with a dirty-yellowish purulent material. The lining of the cavity was formed by tissue similar to that of the greater omentum.

The stomach was moderately distended, and the wall was slightly thickened. The mucosa was injected and purple-brown, and 6.5 cm. above the pylorus, along the lesser curvature, there was an elevated, circumscribed plaque, 11 by 5.5 cm. in the greatest diameter, with two ulcers in the center, 45 and 20 mm. in diameter, respectively, and up to 20 mm. deep. The plaque consisted of a pale yellowish brown homogeneous and moderately firm tissue. The lesser curvature of the stomach was firmly adherent to the left lateral wall of the tumor mass.

On the anterior aspect of the right lobe of the liver, extending close to the capsule, there was a cavity 9.5 by 8 cm. in diameter, lined by a layer of purple-brown tumor tissue, 10 mm. thick. On the inferior aspect of the left lobe of the liver there was a node of light yellow-gray, slightly transparent tumor tissue, 5.5 cm. in diameter.

The right inguinal lymph nodes were enlarged up to 5.5 cm. in the greatest diameter and were composed of a firm glistening pinkish gray tissue.

The remaining organs revealed nothing of particular interest. There was brown atrophy of the myocardium and liver, and the lungs, kidneys and spleen showed passive congestion.

Microscopic Examination.—In many places the tumor of the omentum revealed (fig. 2) thick-walled anastomosing tubes. When one of these tubes was cut transversely, as was frequently the case, the cross section was round or oval and presented a central blood-filled lumen surrounded by a broad cellular coat. The coat was composed of moderate-sized cells, the homogeneous oxyphilic cytoplasm of which showed frayed contours and short processes, which seemed to merge with an intercellular reticulum. The nuclei were oval and possessed well defined chromatin granules, evenly distributed throughout the body of the nucleus (fig. 3). Mitoses were rarely seen. The reticulum varied in extent and thickness. Sometimes it encased small groups of cells or isolated single cells, and in general it became more abundant toward the periphery of the cellular coat. It stained steel blue by the azan method and diamond black with silver. With the former stain one could observe how the orange-red cytoplasm of the cell processes gradually assumed a purple-brown and finally a blue coloration. In the silver-impregnated sections the thicker fibrils became surrounded by a purple-red substance. The central blood-filled space resulted from more spreading apart of the axial portion of the cellular masses, or a definite wide, thin-walled capillary might be found which was lined by endothelium and to the wall of which the reticulum was attached.

The tubes were separated by strands of a more compact tissue which resulted from condensation of the reticulum. The cells decreased in size and number, and the reticulum fibers became thicker, more numerous and fused together, and were surrounded by a ground substance that stained purple-red in the silver impregnated sections. In the sections stained by the azan method, small homogeneous red droplets were visible in the ground substance. These droplets sometimes fused together to form larger, colloid-like deposits. On the free surface the tumor was covered by a definite capsule of fibrillar connective tissue. In some areas the tumor tissue was necrotic. The central vessels were occluded by

mixed thrombi, the cell coats had lost their nuclei and appeared homogeneous, and the structureless tissue then became infiltrated by disintegrating leukocytes.

From this basic type of structure, deviations occurred, the most common one showing a pale bluish (hemalum) stained ground substance with scanty, delicate fibrils. Embedded with this ground substance were cells which closely resembled

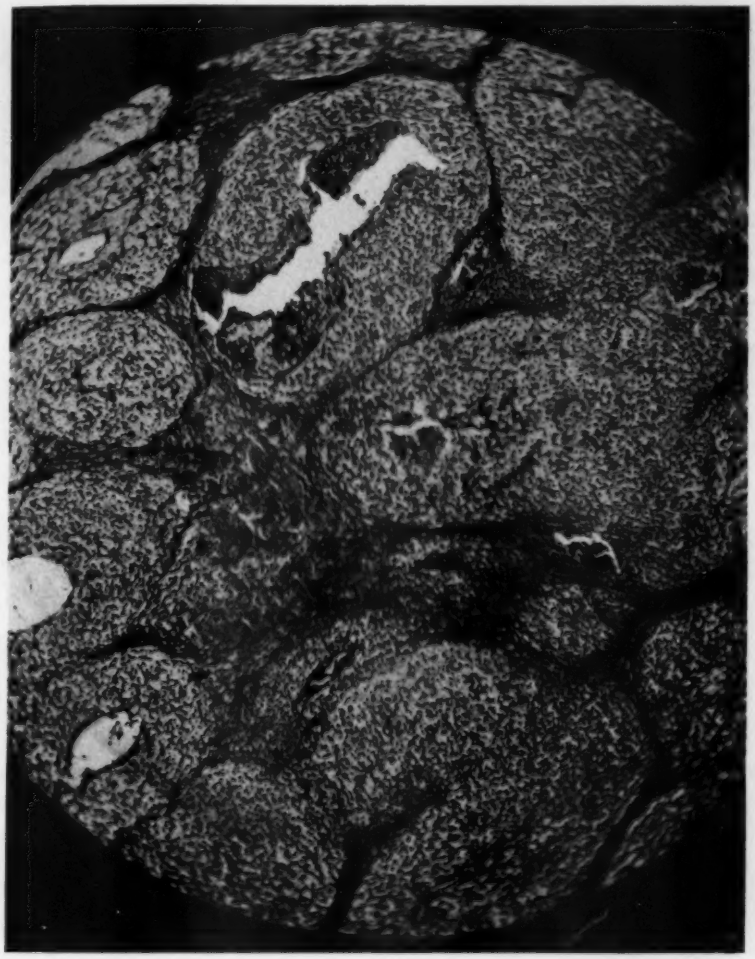

Fig. 2.—Photomicrograph of the tumor of the omentum, showing the organoid glomus-like structures; × 120.

those surrounding the blood vessels. These cells formed rows, usually double rows, or were more diffusely scattered. When a blood vessel penetrated this type of tissue, the cells sometimes had a tendency to arrange themselves about it, and this tendency led to the characteristic organoid formation previously described.

In the nodes attached to the small intestine, which were well circumscribed and which did not affect the intestinal wall except the subserosa in which they

were located, the rows of cells with the pale-stained ground substance predominated. In the periphery one found blood vessels about which the cells tended to form coats.

Each of the metastases to the liver was surrounded by a definite capsule of varying thickness. The capsules fused with the portobiliary septums. The adjacent liver cell cords were slightly compressed and displaced. In structure the metastases

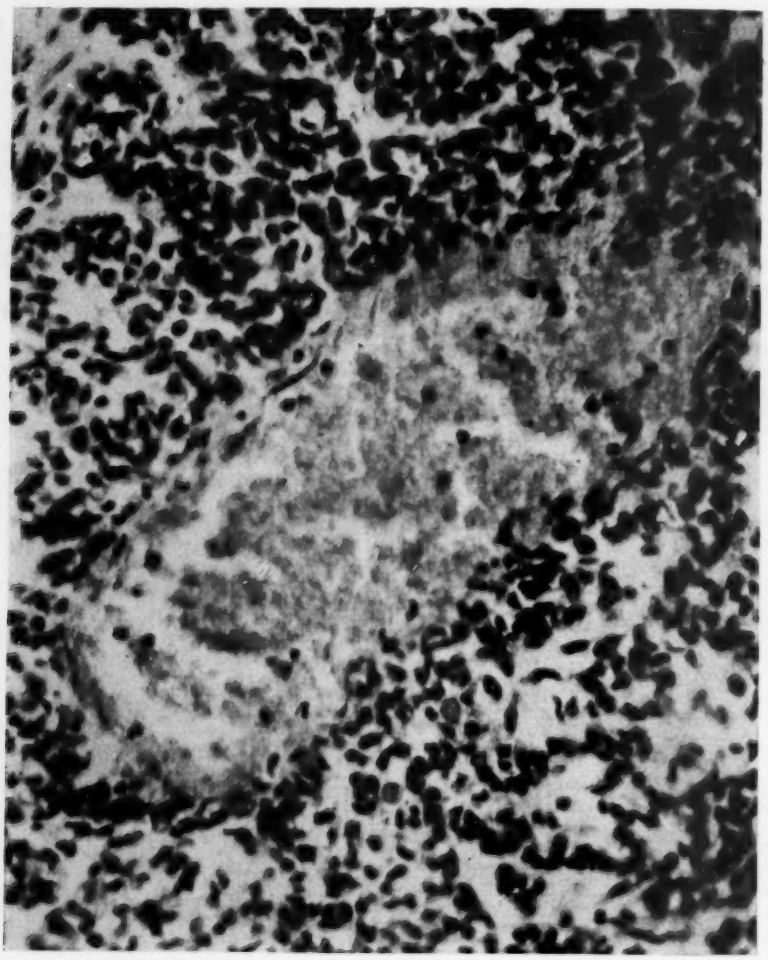

Fig. 3.—Central blood space surrounded by a dense coat of moderate-sized cells containing hyperchromatic oval nuclei; × 300.

essentially resembled the tumor in the omentum. The central liquefaction was preceded by a loosening and vacuolation of the ground substance and dissolution of the nuclei. Throughout the liver the Kupffer cells were much swollen and filled with highly refractile, light gray crystalline inclusions.

Anatomic Diagnosis.—The diagnosis was: primary malignant glomus-like tumor of the greater omentum; metastases to the peritoneum, mesentery, liver, stomach and

right inguinal lymph nodes; brown atrophy of the myocardium and liver; marked emaciation, and pigmentation of the reticuloendothelium with thorium dioxide.

COMMENT

The diagnosis of a primary neoplasm of the omentum is suggested in view of the failure to find a primary site in any of the other viscera in a most careful search. The microscopic appearance of the omentum, as well as the metastases, showed that we were dealing with an unusual type of tumor that had none of the features of a primary malignant growth of the liver, stomach or peritoneum. It was the opinion of the late Dr. R. H. Jaffé that the omentum was the primary site of the tumor, and because of the glomus-like appearance microscopically he agreed that the term "glomangioma" was appropriate.

The two specimens of tumor tissue taken at the operation were puzzling. The first revealed a homogeneous substance with scanty, delicate fibrils, suggesting a malignant neoplasm with origin in the nervous system while the second showed an angiomatous neoplastic tissue not present in the first.

The characteristic appearance of the tumor microscopically was that of an organoid structure resembling an overgrown glomus. This structure was characterized by a central lumen, lined by endothelial cells and filled with blood. The vessels were surrounded by a thick layer of slightly elongated cells of the epithelioid type, as previously described. The single glomus was surrounded and separated by strands of connective tissue.

Normally the omentum contains accumulations of histiocytes in dense masses which are arranged along the blood vessels or at some little distance from them as small or large, round or oval patches, called "milk spots."[35] Do these milk spots have malignant potentialities, and is it possible that our tumor arose from these areas? On the other hand, since undifferentiated mesenchymal cells are found normally scattered in the omental tissue along the blood vessels as pericytes, are we justified in assumming that our tumor may have had its origin from these cells?

Although most of the cutaneous glomus tumors are benign, there are a few rare ones showing a malignant invasive growth. Our metastasizing malignant glomus-like tumor is, in our opinion, unique.

SUMMARY

One hundred and six cases of benign glomus tumors are reviewed, and a case of a malignant tumor of the omentum with features of a glomangioma and metastases is described. It is the first case of a malignant glomus-like tumor of the omentum to be recorded.

35. Maximow and Bloom,[22] p. 87.

PRODUCTION OF RENAL CALCULI IN GUINEA PIGS BY FEEDING THEM A DIET DEFICIENT IN VITAMIN A

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AND

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The etiologic relationship of deficiency in vitamin A to the formation of renal calculi has been the subject of considerable discussion since Osborne, Mendel and Ferry [1] first showed the presence of urinary calculi in rats fed on diets deficient in fat-soluble vitamins. Although numerous investigators have succeeded in inducing urinary lithiasis in rats by employing similar diets, it has become evident that the role of vitamin A in this disease is by no means clear. Simultaneously operating factors, such as improper mineral balance of the diet, the lack of other accessory food factors, excessive alkalinity of the urine and the presence of urinary infection, have been variously implicated by workers who have utilized rats in their studies.

The importance in the diet of disproportionately large amounts of calcium as compared with the amounts of phosphorus has been stressed by McCarrison [2] and by Ranganathan,[3] who succeeded by this means in obtaining formation of calculi in rats despite the administration of vitamin A or of vitamins A and D. Cox and Imboden [4] obtained similar results in their experiments. McCarrison [2] believed that a general deficiency of the diet played a role in the disease since he found that the formation of calculi was more extensive in the animals in which the diet was lacking in vitamin A as well as in the appropriate amounts of phosphorus in relation to calcium. The significance of the mineral content in the diet is indicated by the experiments of Watchorn,[5] who observed that an excess of magnesium carbonate in diets containing

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From the Pediatric Research Laboratory of the Jewish Hospital of Brooklyn.

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4. Cox, W. M., Jr., and Imboden, M.: J. Nutrition **11**:47, 1936.
5. Watchorn, E.: J. Hyg. **32**:156, 1932.

an abundance of vitamin A resulted in formation of urinary calculi in rats. The responsible dietary factors according to Fujimaki,[6] however, are deficiencies of calcium and phosphorus in addition to lack of vitamin A.

Higgins [7] maintained that alkaline urine is in large measure accountable for the urinary calculi which develop in rats fed on diets deficient in vitamin A. He concluded that the increased excretion of base is of such magnitude that the protective action of urinary colloids is no longer adequate to prevent precipitation and stone formation. Finally, it may be mentioned that a number of investigators, including Osborne, Mendel and Ferry,[1] McCarrison [2] and Higgins,[6] have expressed the belief that infection of the urinary tract resulting from dietary insufficiency of vitamin A is at least a contributing factor in the causation of urinary lithiasis.

The selection of rats for studies of experimental urinary lithiasis has not been satisfactory for another reason: Aside from the difficulty of determining the precise role of the deficiency of vitamin A in the experiments outlined, uncertainty is injected by spontaneous formation of calculi in rats. In this connection it has been stated that the rat is "very prone to stone" and that clarification of the problem might be furthered by studying lithiasis in animals normally free from the disease (Joly [8]).

During the course of experimental work on the effect of deficiency in vitamin A on the course of tuberculosis in guinea pigs, Steiner, Greene and Kramer [9] noted that in a number of the animals on the restricted diet renal calculi developed. This was in contrast to the absence of disease of the urinary tract in control series of guinea pigs fed on a variety of diets, including those which lacked other vitamins.[10] These observations suggested that guinea pigs were suitable for the study of the formation of urinary calculi, and experiments which were carried out on nontuberculous guinea pigs form the basis of the present investigation.

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7. Higgins, C. C.: J. Urol. **29**:157, 1933.

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METHODS

The guinea pigs used weighed 300 Gm. and were all obtained from the same farm. They were kept in groups of six in large metal cages, the floors of which were of wire mesh and fitted underneath with removable trays. The animal rooms were large and well ventilated, and the temperature was maintained at from 70 to 80 F.

The basal diet contained the following foods in the percentages given: rolled oats, 20; white corn meal, 41.5; white mashed turnips, 33; brewers' yeast, 3; calcium carbonate, 1.5; sodium chloride, 1, and viosterol, 15 drops per kilogram of ration.

The dry components of the diet were made up weekly. The turnips were ground twice a week, kept in the refrigerator and added to the dry mixture daily in measured amounts. Filter paper clippings were given for additional roughage. Freshly distilled water was given daily, and 1 drop of compound solution of iodine was added to this once a week. The animals were fed 5 cc. of fresh

Number of Animals Surviving at End of a Period of Fifteen Weeks or Longer

	Control Group (Fed Basal Diet Plus Halibut Liver Oil)		Vitamin-Deficient Group (Fed Basal Diet Alone)	
	Animals at Start	Animals Surviving	Animals at Start	Animals Surviving
Absolute number	24	16	36	1
Percentage		66.7±6.5*		2.8±1.9*

Difference in survival = 63.7 + 6.8†

* = Probable error.

† = The difference is more than nine times the probable error.

orange juice six times weekly by pipet. The control animals received 1 drop of halibut liver oil daily as their source of vitamin A. The basal diet produced an alkaline ash of 4 milliequivalents of titratable base per gram of ash.

Thirty-six animals were fed the deficient diet. Twenty-four were fed the control diet. Both groups gained weight until the tenth week, when the deficient animals began to lose weight steadily until death (fig. 1). At the end of fifteen weeks the experiment was terminated. At this time 16 of the 24 control animals were still living, but only 1 of the 36 deficient animals had survived (table).

OBSERVATIONS

Autopsy revealed pneumonia in 28 of the 36 deficient animals and in 2 of 24 control animals. Cloudy swelling of the cornea as described by Boock and Trevan [11] occurred in a single animal. In both groups of animals there was a loss of body fat, but this was more marked in the group deficient in vitamin A.

Urinary Tract.—Pathologic alterations were noted only in the group of animals receiving the vitamin-deficient diet. The most marked

11. Boock, E., and Trevan, J.: Biochem. J. **16**:780, 1922.

change was seen in the ureters, which were found to be enlarged and thickened in 31 of the 35 animals surviving longer than fourteen days. The enlargement occurred in great part as a result of gradual and progressive mucosal thickening, the first evidences of which were visible after about thirty days. In the more advanced stages reduplication of the mucosa occurred, with development of folds or ridges similar to gastric rugae. Desquamation of the epithelium of the urinary tract was a prominent feature in 17 animals. The desquamated epithelium had the appearance of soft white masses, was putty-like in

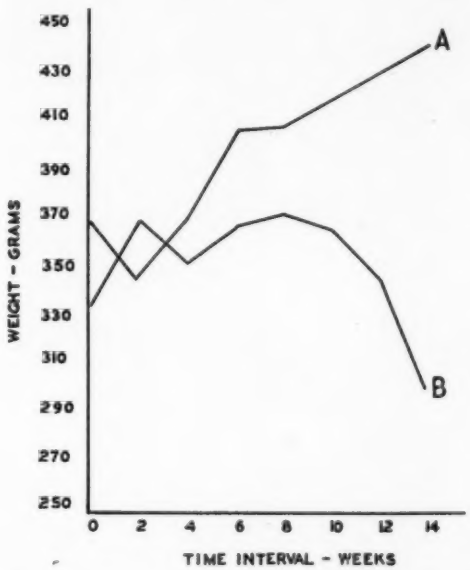

Fig. 1.—Mean average weights of vitamin A–deficient and control animals. *A* represents the average weight of the controls, which were fed ha'ibut liver oil in addition to the basal diet, and *B*, the deficient animals, which received only the basal diet.

consistency and was present in the pelvis and bladder as well as in the ureters. In some instances the desquamated material was attached to the walls of the ureters, but it was easily scraped off. Gross evidence of infection of the urinary tract was absent in all the animals.

Calculi were first observed in an animal which died after having received the deficient diet for fifty-three days. In this animal two small fine calculi were found in a ureter, along with desquamated epithelium. At fifty-eight days calculi were found in one ureter of another animal, following which 7 other animals showed calculi in one ureter or in the

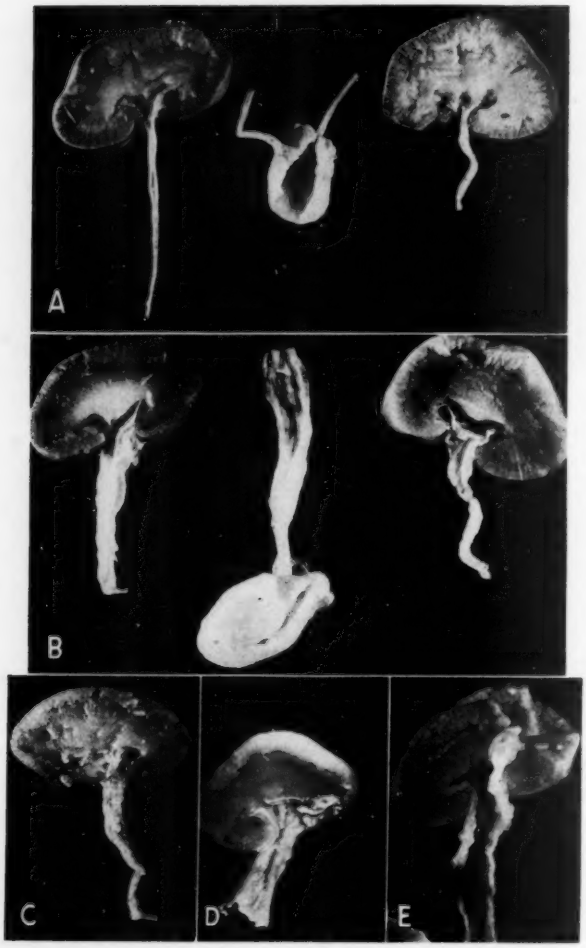

Fig. 2.—*A*, sagittal section showing the pelves, ureters and bladder of a normal guinea pig. This animal was fed on the control diet. *B*, sagittal section showing enlargement of the left kidney (at right) and dilatation and thickening of both ureters and pelves. One small white calculus was found in the left pelvis. The animal was fed the deficient diet fifty-four days. *C*, sagittal sections of a kidney and ureter, showing enlargement and thickening of the ureter. The pelvis of this kidney contained six irregular white calculi. The animal was fed the deficient diet ninety-three days. *D*, sagittal section of a kidney and ureter, showing considerable enlargement and thickening of the ureter. The mucosa was thrown up into folds. The pelvis of the kidney contained soft white masses. The animal was fed the deficient diet ninety-eight days. *E*, sagittal section of a kidney and ureter, showing considerable enlargement and dilatation of the ureter. The pelvis of the kidney was dilated and contained soft white masses. The animal was fed the deficient diet eighty-four days.

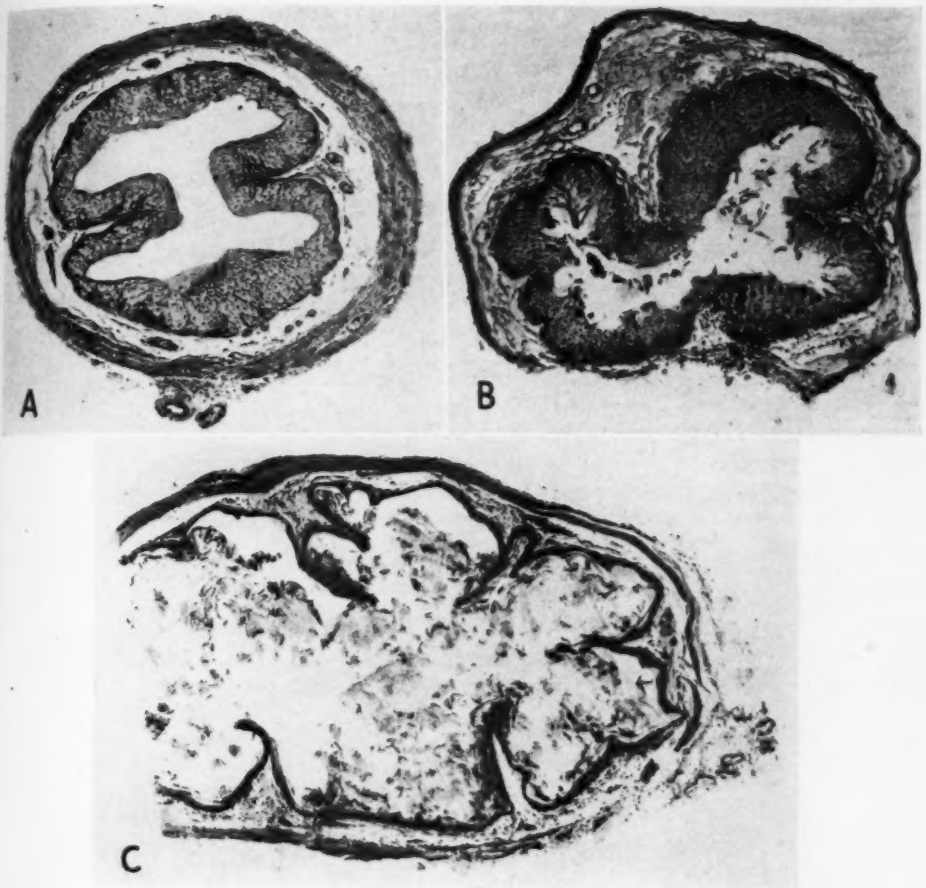

Fig. 3.—*A*, cross section of the ureter of a control animal, showing normal uroepithelium composed of several layers of large hydropic cells; × 74. Note two plical infoldings of the mucosa. *B*, cross section of a ureter, showing enlargement of the ureter, hyperplasia and beginning metaplasia of the mucosa to squamous epithelium; × 74. There is a small amount of desquamation of the epithelium. The animal was fed the deficient diet thirty-nine days. Note absence of infection. *C*, cross section of a ureter, showing considerable dilatation of the lumen, desquamation of keratinized epithelium, atrophic stratified squamous epithelium and many septums, indicating previous hyperplasia of the mucosa; × 74. The animal was fed the deficient diet ninety-eight days.

EXPLANATION OF FIGURE 4

A, longitudinal section of a ureter showing complete replacement of the mucosa by stratified, keratinized epithelium; × 75. Two small ureteral calculi were found in this animal, which was fed the deficient diet fifty-three days.

B, section through the pelvis of a kidney showing calcification of some of the desquamated epithelium and metaplasia of the pelvic mucosa to squamous epithelium; × 75. Both pelves of this animal contained several small irregular white calculi, which were removed before the tissues were fixed. The animal was fed the deficient diet ninety-three days.

C, section through the pelvis of a kidney showing keratinized squamous epithelium and a mass of calculi; hematoxylin and eosin stain; × 75. The kidneys and ureters of this animal were fixed in solution of formaldehyde U. S. P. and sections made with the calculi in situ. The animal was fed the deficient diet sixty-eight days.

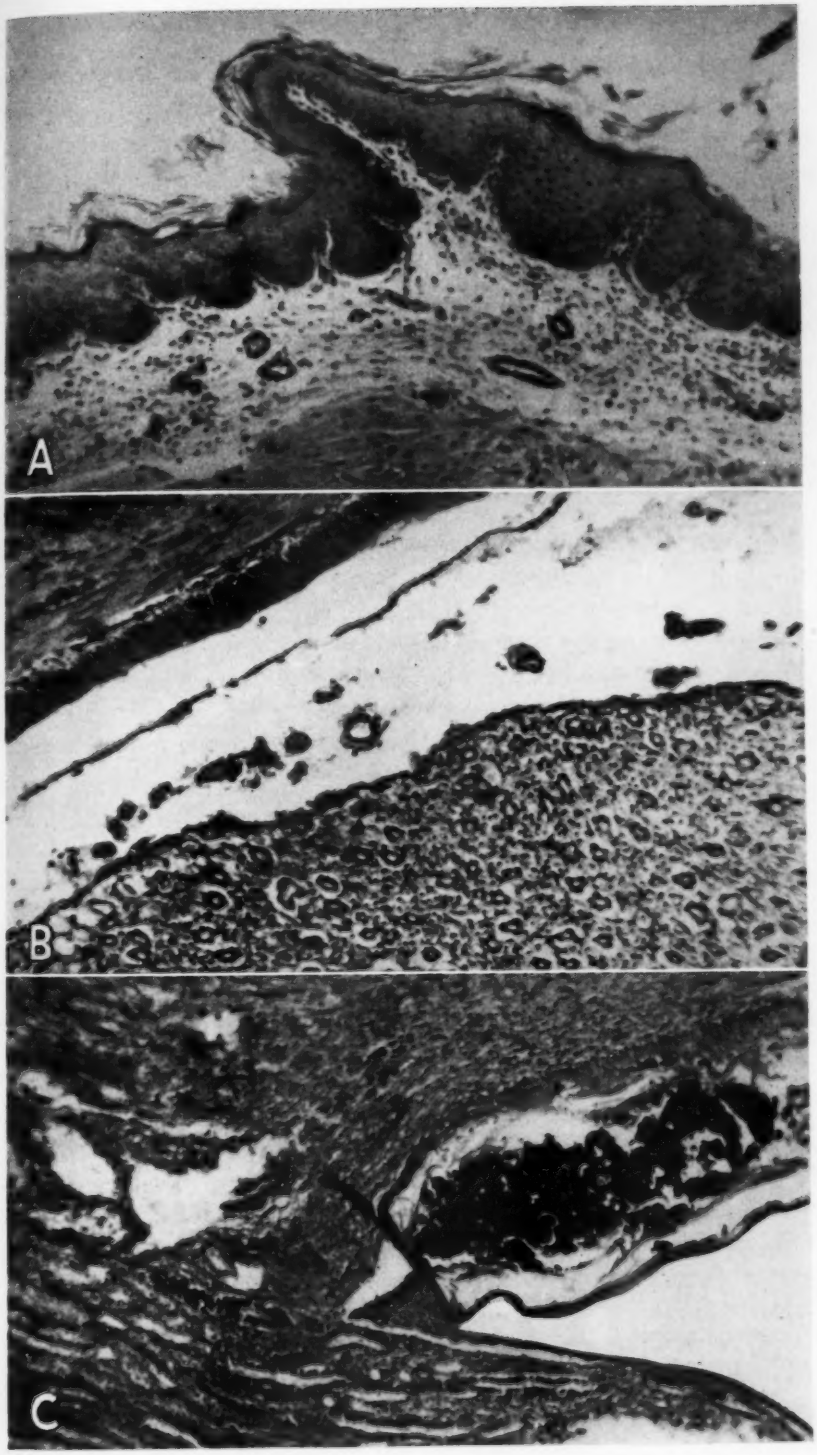

Figure 4

renal pelvis after having received the deficient diet for from eighty to ninty-five days. In 3 of these animals desquamated epithelium was found with the calculi. Thus, in 9 of 35 animals calculi were present in the renal pelvis or ureter. No vesical calculi were found. The calculi varied from pinhead size to a diameter of from 3 to 4 mm. and were dull gray and irregular in shape. The largest number, twelve (six in each pelvis), was found in an animal which had succumbed after ninety-four days on the deficient diet (fig. 2).

The earliest microscopic change appeared to be hyperplasia of the ureteral mucosa, which in the normal guinea pig is made up of several layers of moderately large hydropic cells (fig. 3 *A*). These cells in the deficient animals lost their hydropic appearance, and the number of layers of cells appeared to increase considerably. Desquamation then took place; the cells became more closely packed together, taking on the appearance of stratified epithelium, and became keratinized (figs. 3 *B* and 4 *A*). Subsequent to the desquamation and keratinization of the mucosa, the ureters became dilated. In some animals, in the later stages, the mucosa appeared to be atrophied, and there was much infolding of the basal lining, indicating that earlier there had been considerable hyperplasia of the mucosa (fig. 3 *B*). Cross sections of the ureter of one animal with the calculi fixed in situ showed keratinization of the mucosa with many calcified amorphous masses in the lumen.

The microscopic changes in the renal pelvis were similar to those in the ureter. There was metaplasia of the normal mucosa to stratified keratinized epithelium in the deficient animals. In the pelvis, wherever calculi were found fibrosis was present to some degree, and in several sections calcification of desquamated epithelium could be demonstrated (fig. 4 *B* and *C*). Sections of the ureter and pelvis failed to reveal evidence of infection.

Histologic sections of the trachea likewise showed metaplasia from ciliated columnar epithelium to stratified squamous epithelium and in some cases the mucosa appeared atrophied or completely desquamated.

Analysis of the calculi from 2 animals yielded 24 per cent calcium and 26.8 per cent carbon dioxide. Phosphorus was absent. The theoretic ratio of calcium carbonate is 40 parts of calcium to 44 parts of carbon dioxide. With 24 per cent calcium or 24 Gm. of calcium per hundred grams of material, the theoretic amount of carbon dioxide would be 26.4 per cent. The actual amount obtained was 26.8 per cent, a remarkable agreement, indicating that the inorganic component of the calculi consisted entirely of calcium carbonate.

COMMENT

The possibility of inducing formation of calculi in guinea pigs by feeding them a diet deficient in vitamin A permits a comparison with the conditions thought to be necessary for the formation of calculi in rats. No such comparison has been made before, because no reports of the occurrence of calculi in guinea pigs on a vitamin A–deficient diet have appeared. Wolbach and Howe [12] gave a most complete description of the tissue changes that occur in guinea pigs on a diet deficient in vitamin A, including the histologic sequences in the trachea, salivary glands and uterus. They found keratinizing epithelium in the genitourinary tract but made no mention of finding pelvic or ureteral calculi.

In our experiments on guinea pigs deficiency of vitamin A alone appeared to be the factor responsible for the occurrence of calculi in 9 of 35 animals. The diet of the control animals did not differ from that of the deficient group except in the vitamin A content. Gross evidence of infection in the urinary tract, such as pyonephrosis, was not found in this series of animals. Likewise, microscopic evidence of infection was absent.

Infection in the lungs occurs frequently in guinea pigs and rats on diets deficient in vitamin A and can be attributed to desquamation of the mucosa, plugging of the bronchi and subsequent development of bronchiectasis and pneumonia. In the genitourinary tract the physical conditions are not quite similar to those in the respiratory tract. In the latter tract there is free connection with the exterior, through which bacteria can easily be aspirated.

The one constant observation in the deficient group was the marked epithelial changes in the renal pelves and ureters. We were able to demonstrate calcium deposition in desquamated epithelium of the renal pelvis and in the ureters. The initial changes in the epithelium appeared to be hyperplasia of the normal epithelium and later metaplasia to squamous epithelium, keratinization and finally, in some cases, complete atrophy of the mucosa, especially in the ureters. Large plaques of desquamated epithelial cells were found in the pelvis, ureters and bladder, but calculi were found only in the pelvis and ureters. It appears that these large plaques act as niduses for the formation of the calculi. In our experiments both control and vitamin A–deficient animals came to have alkalinuria, which we attributed to the diet, since analysis of the diet showed an alkaline ash of 4 milliequivalents of titratable base per gram of ash. The vitamin D–deficient group showed a urinary p_H of 8.8, while for the control group the p_H was 8.4. Probably the chief factor in the formation of calculi in vitamin A deficiency is the change in the renal epithelium. Infection apparently plays no part, and alka-

12. Wolbach, S. B., and Howe, P. R.: Arch. Path. **5**:239, 1928.

linization of the urine in itself is not a factor since it was also present in the control animals.

Analysis of the calculi indicated that the inorganic fraction consisted entirely of calcium carbonate. The absence of phosphorus in the calculi is worthy of comment, since conditions which are favorable to the deposition of calcium carbonate usually result in the deposition of calcium phosphate. However, it is possible that because of the minimal amount of phosphorus and the large amount of calcium in the diet, the urinary phosphorus was low. Unfortunately, the urine was not analyzed for phosphorus.

SUMMARY

Renal and ureteral calculi were produced in 9 of 35 guinea pigs by feeding these animals a diet deficient in vitamin A.

The histologic sequences as they occurred in the pelvis and ureter from normal uroepithelium to stratified, keratinized epithelium have been described.

Vitamin A deficiency apparently caused hyperplasia, then metaplasia and finally atrophy of the pelvic and ureteral mucosa. Large plaques of desquamated epithelium acted as niduses for the development of calculi.

The inorganic component of the calculi so formed was calcium carbonate.

There was no gross or histologic evidence of infection in the urinary tract.

INFLAMMATION

XVII. DIRECT EFFECT OF CHANGES IN THE HYDROGEN ION CONCENTRATION ON LEUKOCYTES

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There is definite evidence that the cellular composition of an inflammatory exudate is a function of the concentration of hydrogen ions in the area of inflammation.[1] At p_H above 7 the predominating cell of an exudate is the polymorphonuclear leukocyte. When the p_H falls between 7 and 6.8, the percentage of macrophages exceeds that of polymorphonuclear leukocytes. When the p_H is somewhat lower, all types of leukocytes tend to be injured, and frank suppuration ensues. Thus the hydrogen ion concentration of the exudate appears to be important in determining whether the milieu is favorable for the survival of leukocytes. This fact apparently bears no direct relation to the migratory activity of cells in the inflamed area. The available evidence indicates that the emigration of polymorphonuclear cells in injured tissue is referable to the liberation of a polypeptide-like crystalline substance, which I have termed leukotaxine.[2] The mechanism conducive to local acidosis in an acutely inflamed area seems to be primarily referable to a disturbance in carbohydrate metabolism in the form of enhanced glycolysis.[3] The abundant formation of lactic acid leads to a depletion of the local alkali reserve. This, however, does not necessarily indicate that the fall in p_H is invariably due to production of lactic acid. It is conceivable that with certain types of acute inflammation the augmented concentration of hydrogen ions may be referable to some acids produced by a mechanism other than glycolysis. The studies of Lord[4] suggested that in the development of pulmonary pneumonia a gradual increase in the concentration of hydrogen ions occurs. This investigator conceived resolution as a result of the increased concentration of hydrogen ions, which eventually activates a proteolytic enzyme

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This investigation was aided by grants from the Wellington Memorial Research Fund of the Harvard University Medical School, from the Milton Fund of Harvard University, from the Committee for Scientific Research of the American Medical Association and from the International Cancer Foundation.

1. Menkin, V.: Am. J. Path. **10**:193, 1934.
2. Menkin, V.: J. Exper. Med. **67**:129, 1938.
3. Menkin, V., and Warner, C. R.: Am. J. Path. **13**:25, 1937.
4. Lord, F. T.: J. Exper. Med. **30**:379, 1919.

having a range of optimal reactivity at p_H 6.3 to 5.2. Friedemann and Graeser [5] recently studied the metabolism of lung tissue in experimental lobar pneumococcic infection. They concluded that infected lung tissue manifests a fairly normal aerobic metabolism. Measurements of the concentration of hydrogen ions in the exudate were not taken. This omission renders it difficult to draw any inference as to the possible relation of the cytologic picture in their experiments to the hydrogen ion concentration.

My own earlier findings suggested that the concentration of hydrogen ions conditions the cellular composition of an exudate.[1] When local acidosis failed to develop in an acutely inflamed area, the polymorphonuclear cells were invariably found to persist as the predominating cells. The conclusion was reached that the cytologic picture of an exudate can be predicted with reasonable accuracy from its p_H and vice versa. The data, however, failed to rule out the possibility of a third factor in the interpretation of the observations. In other words, it is conceivable that changes in p_H and the cytologic picture of an inflammatory exudate may constitute two parallel variables. These may show definite correlation, but it is obvious that this fact in itself does not necessarily imply a direct cause and effect relationship. For this reason the present study has been undertaken to determine whether changes in hydrogen ion concentration are per se capable of affecting the viability or activity of leukocytes. It is interesting to note in this connection that a number of years ago Evans [6] was able to demonstrate that these cells are injured by acids. She pointed out that if the injury is not too intense the leukocytes may be restored to a normal condition by merely bathing them in blood serum.[7]

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5. Friedemann, T. E., and Graeser, J. B.: J. Exper. Med. **67**:481, 1938.

6. Evans, A. C.: Pub. Health Rep. **46**:2539, 1931.

7. B. Steinberg and A. Dietz (Arch. Path. **25**:777, 1938) recently alluded to some of the conclusions which I published on the basis of data obtained by an entirely different technic. Their inferences are definitely open to question for the following reasons: With blind introduction of glass electrodes into the peritoneal cavity there is no proof that during the period of experimentation the electrodes are constantly in contact with an adequate amount of exudate. The mobility of the viscera accompanying breathing is likely to shift the electrodes or the amount of fluid in which they are dipped, thus favoring variations in readings. Furthermore, it is questionable whether measurements under anesthesia may not induce modifications of the true p_H of an exudate. The cytologic studies and the p_H measurements could not have been performed by Steinberg and his co-worker on identical samples of exudate. This tends to invalidate any inferences regarding the exact correlation of the type of cells to the hydrogen ion concentration of a given sample. In brief, it is conceivable that the electrodes as placed in the cavity were frequently measuring the hydrogen ion concentration of the lining membranes,

EXPERIMENTS

Inflammatory exudates were obtained by introducing from 1 to 2 cc. of turpentine into the right side of the chest in dogs.[3] The irritant was administered under anesthesia obtained with pentobarbital sodium. On the following day a sample of the exudate was withdrawn by means of a Luer syringe with a hypodermic needle. The latter was of large caliber and filed off at the end in order to diminish the chance of injury to the lung.

A series of slides were stained with neutral red in accordance with Sabin's[8] technic. Two drops of a standard buffer followed by a drop of the exudate was deposited on a slide. The mixture was sealed by covering the edge of the coverslip with a melted petrolatum paraffin preparation. The slides were incubated at 37 C. for varying periods.

The standard solutions used as buffers were prepared according to the directions of Sørensen and of Cohn[9] by mixing 0.0667 molar potassium dihydrogen phosphate with varying concentrations of disodium hydrogen phosphate. The p_H values obtained on the addition of 1 part of exudate to 2 parts of the standard buffers ranged between 7.6 and 6. To determine whether the cells of an exudate were especially sensitive to lactic acid, different concentrations of this acid were prepared in a buffer mixture having an initial p_H of 7.35. The final p_H of these lactic acid solutions when in contact with exudate ranged between 7.2 and 5. The p_H standards selected were such as to approximate the levels of hydrogen ion concentration encountered in various stages of an inflammatory reaction.[10] Control

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whereas the cellular studies were actually made on a sample of exudative material. The inconsistent variations and shifts obtained by these investigators when values of p_H were compared both by in vivo and in vitro measurements cast further doubt not so much on the possible escape of the gaseous contents of the sample (incidentally, this is easily obviated when the sample is removed and studied under oil) as on the validity of their technic. Finally, as pointed out by A. B. Hastings and J. Sendroy (J. Biol. Chem. **61**:695, 1924), the colorimetric method employed by me has been checked against a potentiometric method and found to be perfectly satisfactory. This, in addition to factors such as the "protein error," has previously been discussed by me.[1] The measurements recorded by a large number of investigators, particularly those of H. Schade (Die Molekularpathologie der Entzündung, Leipzig, Theodor Steinkopff, 1935) and his school, who have used electrometric methods, all indicate that acute inflammation is conducive to local acidosis. In addition to the studies reported by me and by others, an investigation by Menkin and Warner,[3] which Steinberg failed to take cognizance of, substantiated further the earlier data on the increased concentration of hydrogen ions by showing that the increase is primarily referable to an increase in lactic acid produced on the development of an acute inflammatory reaction. It is also perhaps worth while to point out the rather obvious admonition that before assuming the absence of any relationship between the cellular composition and the concentration of hydrogen ions in an exudate, precisely the same types of technical experiments should be made on dogs as were performed originally by me, experiments which led me to conclude that there was a definite relationship. Not only have these experiments and conclusions been repeatedly confirmed by me, but M. B. Lurie (Am. J. Path. **13**:612, 1937), employing the same technic with guinea pigs, has arrived at precisely the same conclusions.

8. Sabin, F. R.: Bull. Johns Hopkins Hosp. **34**:277, 1923.
9. Cohn, E. J.: J. Am. Chem. Soc. **49**:173, 1927.
10. Menkin.[1] Menkin and Warner.[3]

smears were likewise set up. These consisted of exudate alone and of exudate in contact with 2 parts of physiologic solution of sodium chloride.

After the preparations had been incubated for from one to two hours, the cells were examined. The criteria employed for the detection of cell damage included changes in: activity, structural appearance, staining quality, extent of cytolysis and of vacuolation, and agglutinating tendencies. A number of such experiments were set up. For convenience a type protocol summarizing the results of an experiment is given in the accompanying table.

The data in this protocol indicate that the polymorphonuclear leukocyte in contact with an acid medium manifests definite signs of injury. The cell becomes nonmotile. It tends to become round. The dye either fails to stain the cytoplasmic granules or penetrates exclusively within the nucleus. Staining of the nucleus by a vital dye is a well known sign of cellular injury. The leukocyte tends to

Protocol on the Exudate of a Dog

p_H of Exudate	Motility of Polymorphonuclear Leukocytes*	Staining Reaction	Swelling of Cells	Large Vacuoles and Coarse Granules	Clumping of Cells	Cytolysis
7.60	+	+	0	0	0	0
7.28	+	—	0	0	0	0
6.60	0	Many stained nuclei	+	+	+	+
6.00	0	Many stained nuclei	+	+	+	+
7.50†	+	+	0	0	0	0
7.23†	+	+	0	0	0	0
5.40†	0	0‡	+	+	+	+
5.00†	0	0‡	+	+	+	+
7.20 (saline control)	+	+	0	0	0	0
7.30 (exudate alone)	+	—	0	0	0	0

* In this and subsequent columns a plus sign indicates either the presence or the normal appearance of the factor studied; a zero, its absence.

† This is the final p_H obtained by mixing exudate with varying dilutions of lactic acid in a standard buffer at p_H 7.35.

‡ Several cells contained nuclei that had absorbed dye.

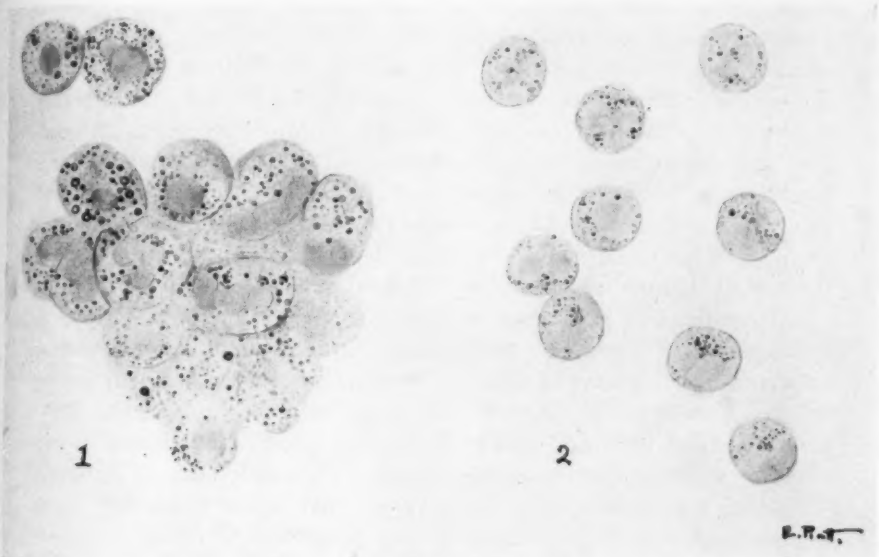
swell, and at times exhibits numerous protoplasmic protrusions at the periphery. The cytoplasm presents a coarsely granular appearance; newly formed vacuoles may likewise form a conspicuous feature of the reaction. Adherence or clumping of leukocytes usually occurs. In such agglutinated masses of cells, signs of protoplasmic disintegration and of cytolysis are apparent. The in vitro effect of an acid and of an alkaline reaction on polymorphonuclear leukocytes is illustrated in the figure (*1* and *2*, respectively).

OBSERVATIONS

The observations presented in this report indicate that polymorphonuclear leukocytes are definitely injured when in contact with an acid medium with p_H about 6.6 or below. The p_H level at which signs of cellular injury are detected by this in vitro method approximates reasonably well the hydrogen ion concentration found in inflammatory exudates containing an abundance of degenerated polymorphonuclears. In view of this fact it is plausible to suppose that the failure to find

normal polymorphonuclear leukocytes in the late stages of an acute inflammatory reaction is referable to the developing local acidosis.[10] There is no evidence that lactic acid per se exerts a specific deleterious effect. The degree of acidity, irrespective of its origin, seems to be the determining factor. The present study serves as further evidence in support of the view that the cytologic composition of an inflammatory exudate is conditioned by its hydrogen ion concentration.

The observations made demonstrate the sensitiveness of polymorphonuclear leukocytes, the initial type of cell in inflammation, to the action of acids. Previous studies have definitely indicated that macro-



1, a group of supravitally stained polymorphonuclear cells from an exudate in contact with an acid buffer standard. The final p_H is about 6.5. Note signs of cellular injury. The leukocytes appear as a cluster of swollen, coarsely granulated cells with stained nuclei.

2, cells obtained from the same exudate as those in *1* but exposed to a hydrogen ion concentration of about p_H 7.4. In sharp contrast to the injured cells exposed to the acid reaction there is now no sign of cellular aggregation; the staining of the cytoplasmic granules is normal, and there is no dye in the nuclei.

phages appear to be more resistant than polymorphonuclears to the action of an increased concentration of hydrogen ions.[1] Contrary to its effect on granulocytes, the reaction at p_H 6.8 seems to be practically innocuous to macrophages. At lower p_H, however, as encountered in the later or more intense stages of inflammation, macrophages likewise appear injured, and frank suppuration results. Several experiments

were set up to determine the effect on macrophages of varying the p_H level.

White mice were given an intraperitoneal injection of about 0.5 cc. of olive oil or 2 cc. of paraffin oil. About one to two days later the animals were killed and the peritoneal cavities exposed. With varying frequency, small amounts of viscous exudate were collected from the peritoneal cavities. One drop of this material suspended in 2 drops of various standard buffers (p_H 5.5 to 7.4) was studied by the supravital technic.

The cells in such specimens were found to be practically all macrophages. The p_H of the untreated exudate was definitely in the acid range, being about from 6.5 to 6.6. A number of the cells appeared swollen and were apparently laden with oil droplets. Samples of exudate treated with standard buffers with p_H ranging between 6.9 and 6.5 showed after incubation at 37 C. neither morphologic nor staining abnormality. The cells appeared essentially uninjured. At p_H 5.5, however, the macrophages displayed striking impairment. The conspicuous features can be listed as follows: manifest cellular swelling, frequent clumping, coarse vacuolation (with prominent brownian motion of granular material), indications of cytolysis, absence of dye in cytoplasmic structures and occasional diffuse pinkish staining of nuclei. Consequently it is clear that when subjected to a marked increase in hydrogen ion concentration macrophages, as well as polymorphonuclear cells, manifest evidence of injury. The p_H reaction at 6.5 to 6.8 seems, on the other hand, to be relatively innocuous to macrophages. Polymorphonuclear cells are, as has been shown, definitely injured when exposed to such slightly acid mediums. This difference in behavior indicates a greater resistance on the part of macrophages to a developing acidosis. The results presented in this report are therefore in agreement with the view that in an area of acute inflammation the concentration of hydrogen ions apparently conditions the cytologic picture.[10]

CONCLUSIONS

In vitro observations are reported of the effect on leukocytes of changes in hydrogen ion concentration. An acid reaction of p_H 6.6 or thereabout is distinctly deleterious to polymorphonuclears. The extent of cellular injury is manifested by absence of motility, abnormal staining reaction, swelling, vacuolation, clumping and cell disintegration. Macrophages are apparently undamaged in a medium with p_H ranging between 6.5 and 6.8. At lower p_H, however, such as 5.5, these cells are distinctly injured.

The present evidence supports, furthermore, the view that the viability of leukocytes in an acutely inflamed area is a function of the

local concentration of hydrogen ions. The macrophages are more resistant than the polymorphonuclear cells to changes in hydrogen ion concentration. The replacement of polymorphonuclear cells by macrophages in the course of inflammation is reasonably explained on the basis of a development of acidosis in the inflamed area. With further increase in the local concentration of hydrogen ions, both types of leukocytes are actively affected, and a state of suppuration follows.

CARDIAC MUSCLE IN IDIOPATHIC HYPERTROPHY OF THE HEART IN INFANCY AND IN NORMAL GROWTH

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AND
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Karsner, Saphir and Todd [1] counted and measured the fibers in a normal heart, a hypertrophic heart and an atrophic heart and demonstrated beyond reasonable doubt that there is no hyperplasia of the fibers in hypertrophy of the heart in adults.

The problem of idiopathic hypertrophy of the heart [2] in infancy is somewhat different, because at this age many cells possess potentialities of mitotic division which are lost in later life.

Recently McMahon [3] demonstrated a few mitotic figures in cases of idiopathic hypertrophy and suggested that hyperplasia of muscle fibers occurs. The method of Karsner, Saphir and Todd [1] offers an approach to this problem by which more reliable results may be obtained.

THE NUMBER OF FIBERS AND OF NUCLEI IN THE CARDIAC MUSCLE OF AN INFANT WITH IDIOPATHIC HYPERTROPHY OF THE HEART

A postmortem examination was made by Dr. Walter L. Herrmann, at the Babies and Children's Hospital, Cleveland, on a 53 day old colored girl with typical idiopathic hypertrophy of the heart. The infant had shown moderate anemia and pyelitis. Otherwise the clinical history and physical findings were indefinite. Death occurred suddenly, with cyanosis and dyspnea. The heart weighed 52 Gm. The ductus arteriosus and foramen ovale were patent but not to a greater extent than would be expected at this age.[4] Otherwise the heart showed no

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1. Karsner, H. T.; Saphir, O., and Todd, T. W.: Am. J. Path. **1**:351, 1925.

2. Stoloff, E. G.: Am. J. Dis. Child. **36**:1204, 1928. Kugel, M. A., and Stoloff, E. G.: ibid. **45**:828, 1933. Putschar, W.: Beitr. z. path. Anat. u. z. allg. Path. **90**:222, 1932. Pompe, J. C.: Ann. d'anat. path. **10**:23, 1933.

3. McMahon, H. E.: Am. J. Dis. Child. **55**:93, 1938.

4. Patten, B. M.: Am. J. Anat. **48**:19, 1931. Scammon, R. E., and Norris, E. H.: Anat. Rec. **15**:165, 1918-1919.

pathologic change, and the only changes found in the other viscera were chronic passive hyperemia of the liver, spleen and kidneys and fatty metamorphosis of the liver.

Microscopically the heart showed slight edema of the interstitial tissue but no cellular infiltration. The fibers were large, but there was no evidence of vacuolation as seen in cases of glycogen storage disease.

As a control, the heart from a 50 day old colored girl of approximately the same height and weight was selected and prepared in the same manner. Its weight was 25 Gm., which is an average normal weight according to the figures of Coppoletta and Wolbach.[5]

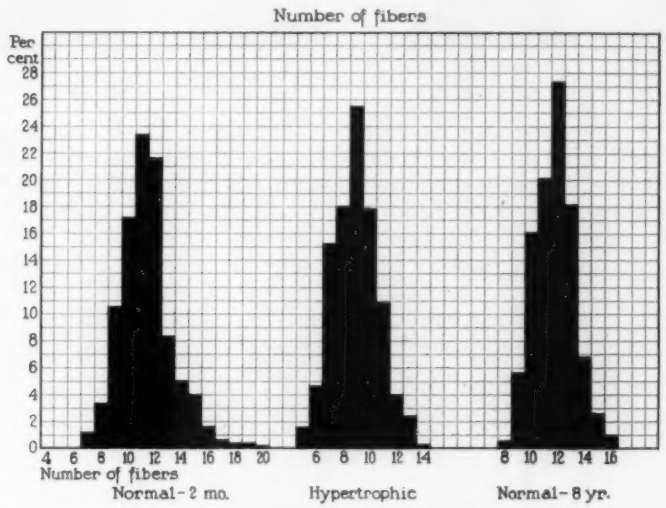

Chart 1.—The percentage distribution of the number of fibers in a standard microscopic field in the normal heart of an infant 2 months old, in the normal heart of a child 8 years old and in the heart of an infant 2 months old with idiopathic hypertrophy of the heart.

Method of Study.—Blocks were cut from the anterior wall of the left ventricle at the level of the papillary muscle and embedded in paraffin. The tissues from the two hearts were treated with the same reagents at the same time in order to equalize the effects of shrinkage. Sections were cut at 7 microns and stained with hematoxylin and eosin. In each heart 500 fields were counted in the manner described by Karsner, Saphir and Todd.[1] In a determination of the breadth of the fibers, only those fibers were measured which had parallel cell walls and which exceeded 175 microns in length. All measurements were made at the level of the nucleus.

Results.—The results are shown graphically in charts 1, 2 and 3 and the statistical analyses in table 1.

5. Coppoletta, J. M., and Wolbach, S. B.: Am. J. Path. **9**:55, 1933.

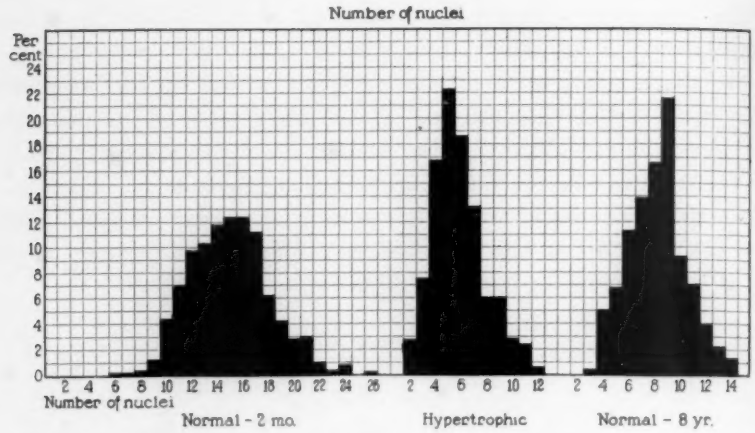

Chart 2.—The percentage distribution of the number of nuclei in a standard microscopic field in the normal heart of an infant 2 months old, in the normal heart of a child 8 years old and in the heart of an infant 2 months old with idiopathic hypertrophy of the heart.

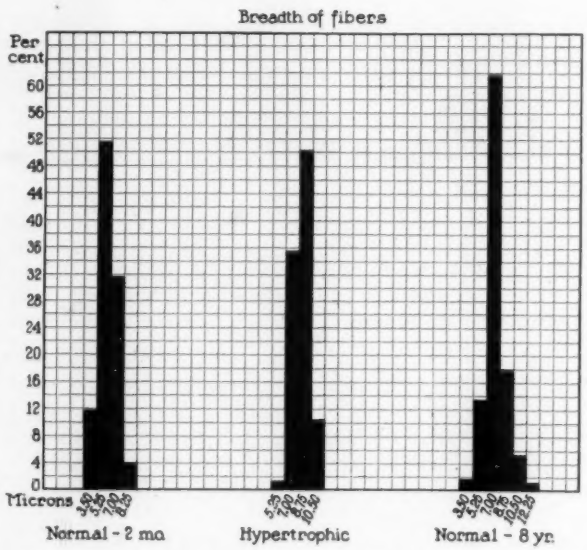

Chart 3.—The percentage distribution of the breadth of the muscle fibers in the normal heart of an infant 2 months old, in the normal heart of a child 8 years old and in the heart of an infant 2 months old with idiopathic hypertrophy of the heart.

As found by Karsner, Saphir and Todd,[1] there is greater variability of the numbers of nuclei and fibers in the normal than in the hypertrophic heart, but the breadth shows about the same variation in both.

TABLE 1.—*Statistical Analyses for the Hearts of a Normal Infant and an Infant with Idiopathic Hypertrophy of the Heart*

Heart	Maximum	Minimum	Mean	Standard Deviation
Number of Fibers per Field				
Normal	20	7	11.14	3.3
Hypertrophic	14	5	8.93	1.7
Number of Nuclei per Field				
Normal	26	6	15.01	2.44
Hypertrophic	12	2	5.79	2.04
Breadth of Fibers in Microns				
Normal	8.75	3.50	5.74	1.26
Hypertrophic	10.50	5.25	8.12	1.21

The density of the muscle in these hearts is not available, but if the average figure of 1.025 is taken,[1] the total numbers of fibers and nuclei may be calculated as follows:

Normal Heart

Weight =25 Gm.; volume =24.4 cc.
Averages=11.14 fibers in 0.000062 cu. mm.
15.01 nuclei in 0.000062 cu. mm.
Totals =4,380,000,000 fibers in 24.4 cc.
5,890,000,000 nuclei in 24.4 cc.

Hypertrophic Heart

Weight =52 Gm.; volume =50.2 cc.
Averages= 8.93 fibers in 0.000062 cu. mm.
5.79 nuclei in 0.000062 cu. mm.
Totals =7,230,000,000 fibers in 50.2 cc.
4,670,000,000 nuclei in 50.2 cc.

These findings are in sharp contrast with those of Karsner, Saphir and Todd [1] for the adult heart on several points: First, in hypertrophy of the heart in an infant there is an apparent numerical increase in the number of fibers; second, there is no increase or perhaps even a slight decrease in the number of nuclei in the same condition, and, third, there are in the normal infant's heart only one-fifth as many fibers and nuclei as in the normal adult's heart.

The figures for the hearts of the normal adult and infant should be comparable because the methods of investigation were as nearly identical as possible. These results force the conclusion that there is an increase in the number of fibers and the number of nuclei in the normal growth of the heart.

The increase in the number of fibers without an increase in the number of nuclei is more difficult of explanation. If the heart muscle is a syncytium it is possible that the fibers can branch without a multiplication of nuclei and give an increase in fibers alone as counted by this method. It is also possible that an increase in the length of the individual fibers is responsible for the increase in the number of fibers counted. No mitotic figures have been seen as reported by McMahon.[3] It is possible that if the cause of the hypertrophy were operative over a longer period than two months of extrauterine life, as in this case, an increase in the number of nuclei would occur.

With these conclusions, it becomes important to count the number of fibers and the number of nuclei in a normal heart from a child so as to fill the gap between the infant and adult values and confirm or refute the inferential conclusion that there is an increase during normal growth.

TABLE 2.—*Statistical Analyses for the Heart of a Child Eight Years Old*

	Maximum	Minimum	Mean	Standard Deviation
Number of fibers per field	16	8	11.71	1.50
Number of nuclei per field	14	3	8.14	2.26
Breadth of fiber, microns	12.25	3.50	7.22	1.63

THE NUMBER OF NUCLEI AND OF FIBERS IN THE CARDIAC MUSCLE OF A CHILD EIGHT YEARS OLD

The heart from a boy 8 years old who died of acute appendicitis and generalized peritonitis was fixed in solution of formaldehyde U. S. P., and paraffin sections were prepared as outlined in the foregoing section of this paper. The heart weighed 120 Gm. and showed no gross or microscopic change. Counts and measurements of this heart are shown in the charts and the statistical analyses in table 2.

On the basis of a determined specific gravity of 1.078 (83 Gm. of ventricular muscle equals 77 cc.) the total number of fibers and of nuclei may be calculated as follows:

Weight =120 Gm.; volume =111.2 cc.
Averages=11.71 fibers in 0.000062 cu. mm.
8.14 nuclei in 0.000062 cu. mm.
Totals =21,000,000,000 fibers in 111.2 cc.
14,600,000,000 nuclei in 111.2 cc.

From a comparison of the numerical data given for the infant, child and adult, it is clear that the heart may increase in size by an increase in the width of the fibers, by an increase in the length of the fibers and by an increase in the numbers of fibers and nuclei.

During normal growth all of the factors are operative, so that there is an increase in the width and length of the fibers and in the number of the nuclei. However, the increase in length occurs more rapidly than the increase in number of nuclei, as shown by the fact that at 8 years the number of fibers approaches the value for the adult heart, while the number of nuclei and the width of the fibers at 8 years are midway between the number and the width in infancy and the number and the width in adulthood.

These conclusions are consistent with the observations in hypertrophy of the heart. In infancy the first response of the hypertrophying heart is an increase in the length of the fibers as shown by a 65 per cent increase in the number of fibers counted by this method. Associated with this increase in length there is a 42 per cent increase in width but no increase in the number of nuclei. In the adult heart, according to the figures of Karsner, Saphir and Todd,[1] there is no increase in the number of fibers or in that of nuclei but an 80 per cent increase in the width of the fibers. Thus the heart of the infant undergoes hypertrophy by an increase in the length and width of the fibers, while the heart of the adult undergoes hypertrophy by an increase in the width only. In neither is there an increase in the number of nuclei, although it is possible that a study of a hypertrophic heart in an infant older than 2 months would show a numerical increase of the nuclei.

SUMMARY

In a case of idiopathic hypertrophy of the heart in an infant 53 days old the apparent number of muscle fibers was increased above the normal but there was no increase in the number of nuclei.

A study of the hearts of an infant 2 months old, a child 8 years old and an adult indicates that there is an increase in the total number of fibers and in that of nuclei during normal growth.

Case Reports

POSTMEASLES ENCEPHALITIS

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In recent years encephalitis occurring in the course of or after measles has attracted more and more attention. In measles of the more severe or fulminating type the encephalitis may have been overlooked, since it is not unusual to have symptoms of nervous irritation in infectious diseases. In the last decade the complication has received attention from Musser and Hauser,[1] Ferraro and Scheffer,[2] Zimmerman and Yannet[3] and Greenfield,[4] none of whom, however, was certain that it was caused by the specific virus of measles. Until the encephalitis can be produced experimentally, the etiologic character will remain in doubt. Today, three views are held: that the encephalitis is due to the virus of measles, that it is an allergic or anaphylactic phenomenon and that it is caused by an unknown virus separate and distinct from that of measles. Although all of these standpoints receive some support, no one of them has been definitely established.

The significance of the case now reported lies in the fact that the patient lived for eight months following the attack of measles. Consequently we have studied a brain in which the disease process had acted over a considerable period. In most of the previously reported cases the patient lived but a short time after the onset of the encephalitis.

REPORT OF CASE

A white boy aged 5 years had an attack of measles six months before his admission to the hospital. About four weeks after this attack the mother noticed that the child was nervous, complained of severe headaches and suffered slight fainting spells and occasional vomiting. She then brought the child to a local hospital, where the diagnosis of meningitis was made. The boy was soon sent home, but about three days later he limped with his right leg. He still complained of severe headaches and gradually lapsed into a semicomatose state.

The boy was well developed and nourished. He lay quietly in bed and appeared acutely ill. Both knees were drawn up, and he was sweating profusely. He did not respond to any questions but cried out whenever he was touched or moved. The pupils were widely dilated and did not react to light. The nose and ears were normal. The tongue was coated, and the pharynx was injected. Over

From the departments of pathology of the Tulane University School of Medicine and the State Charity Hospital of Louisiana.

1. Musser, J. H., and Hauser, G. H.: J. A. M. A. **90**:1267, 1928.
2. Ferraro, A., and Scheffer, I. H.: Arch. Neurol. & Psychiat. **25**:748, 1931.
3. Zimmerman, H. M., and Yannet, H.: Arch. Neurol. & Psychiat. **24**:1000, 1930.
4. Greenfield, J. G.: Brain **52**:171, 1929.

the entire chest resonance was normal. The breath sounds were clear, and the heart sounds were normal but rapid. The abdomen did not reveal anything abnormal. Kernig and Brudzinski signs were present, and the deep reflexes were active. The spinal fluid on admission was clear and under normal pressure, and the cell count was 40, all the cells being lymphocytes. The globulin was 4 plus, and the sugar was 33 mg. per hundred cubic centimeters of fluid. Three days later the chlorides of the spinal fluid were 655.2 mg. per hundred cubic centimeters, and the sugar 62 mg. Twenty days later another sample of spinal fluid showed the cells to be less than 10 and the globulin to be normal. Sixteen days later the last examination of spinal fluid showed 45 lymphocytes and globulin 3 plus.

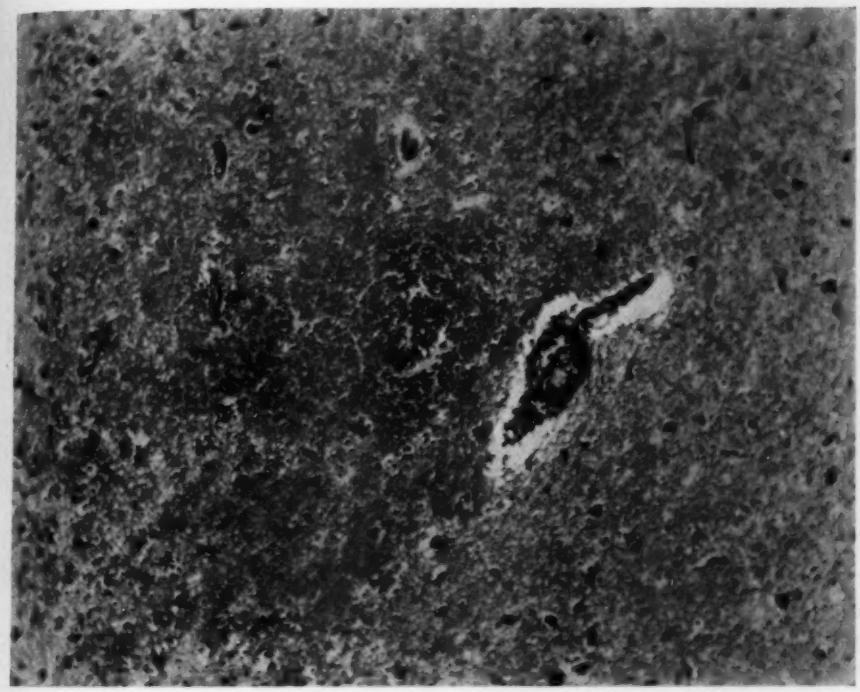

Fig. 1.—Perivascular glial proliferation in the white substance; Nissl stain.

Smears and cultures made of these samples of the spinal fluid were all negative. Roentgen examination of the chest showed clear lung fields and that of the skull some density of the left mastoid region. The urine was normal. The total white cell count was 7,600 and the hemoglobin 80 per cent. Shortly after admission it was noted that the boy had bilateral optic atrophy and was almost totally blind. One week after admission pus began to run from the left ear. Mastoidectomy on the left side was done and both temporal lobes were explored by means of a Cushing needle, but no pus was found. The temperature now ran up to 105 F. and remained high. Death occurred ten weeks after admission.

Autopsy.—The body was that of a well developed and nourished boy approximately 5 years of age, weighing about 45 pounds (20.4 Kg.) and measuring 115 cm. in length. The wound of the recent mastoidectomy was clean and

appeared to be healing well. In each temporal region was an incision 1.5 cm. in length closed with black silk. The pupils were equal, regular and 4 mm. in diameter. No discharges were noted in the ears, nose or mouth. The rest of the body appeared normal.

When the scalp was reflected, two openings were noted in the temporal bones just beneath the previously described incisions of the scalp each of which measured 1 cm. in diameter. The bone of the calvarium appeared thin. The dura mater was normal. The brain was large. No abnormalities were seen in the circle of Willis or in the dural sinuses. The leptomeninges were delicate and clear, and the pial vessels showed considerable congestion. There was a large amount of sub-

Fig. 2.—Special stain of the perivascular proliferation, demonstrating that the cells are for the most part microglia cells; Hortega's silver carbonate method.

arachnoid fluid, especially at the base of the brain. The gyri were flattened, the sulci narrowed, and the whole brain appeared edematous. The cortical gray matter was of normal width and sharply demarcated from the white matter. The blood vessels, especially those in the white matter, were markedly congested. The white matter about the lateral ventricles was very soft. The lateral, third and fourth ventricles were dilated and filled with a slightly yellow clear fluid. The ependyma was smooth.

The heart muscle was pale brown and its density markedly decreased; the valves were normal. In the posterior portion of both lower lobes of the lungs was some congestion. The liver revealed throughout the cut surface numerous focal yellow areas. The spleen was soft, and the pulp was pink. The pancreas, adrenal glands,

gastrointestinal tract, gallbladder, kidneys, urinary bladder and genital organs did not show any gross abnormalities.

Microscopic Examination.—The meninges were considerably congested. There was no trace of exudate.

The brain was hyperemic. The vascular endothelium was hyperplastic or swollen in many instances. Many cortical nerve cells had undergone degeneration. Some nerve cells showed shrinkage of the cell body, disappearance of the Nissl granules and deformity of the nucleus. The most characteristic changes were in the white matter, where cellular proliferations were found, which were most numerous around the blood vessels (fig. 1) but which spread out in smaller

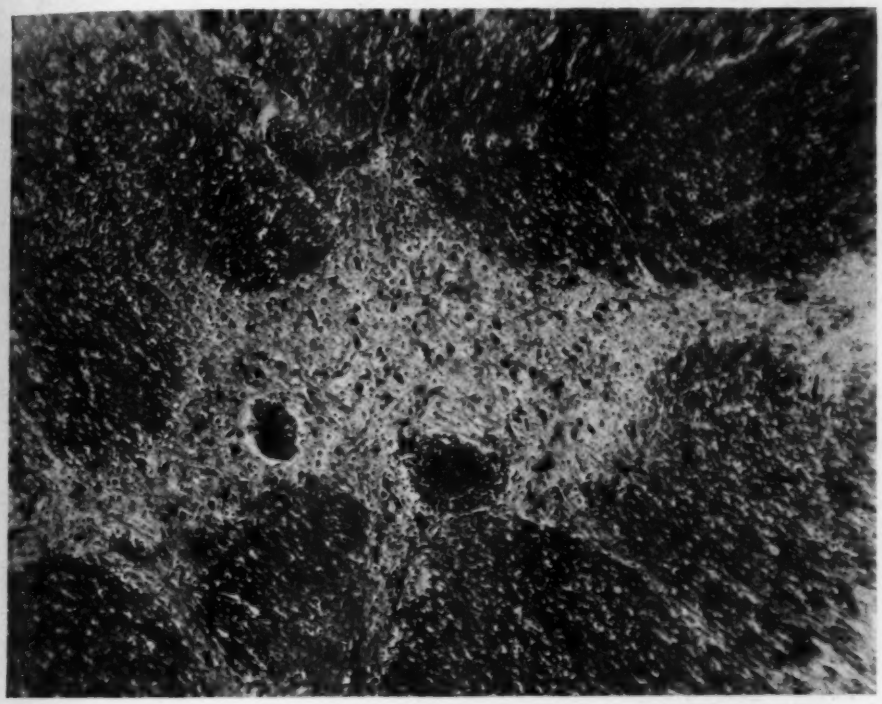

Fig. 3.—Areas of demyelinization about blood vessels; iron-hematoxylin stain.

numbers for variable and often considerable distances into the adjacent tissues. Numerous foci of various sizes, composed of these cells, were scattered throughout the white substance. This proliferation consisted mostly of glia cells with a slight admixture of lymphocytes and plasma cells. Most of the glia cells were microglia (fig. 2), and in some instances these cells showed transformation into larger elements up to compound granular corpuscles.

Special stains for myelin sheaths revealed numerous patches of demyelinization (fig. 3), which were most characteristic in the perivascular regions. Fat stains showed practically no fat substance in the areas of demyelinization, but some fat droplets were seen free or within large phagocytic cells.

The process described was found in all areas of the white substance. In parts of the basal ganglions, also, lesions of a proliferative character were seen.

COMMENT

This case presents a fairly characteristic pathologic picture of the encephalitis of measles, one coinciding with the descriptions given by other authors. The most prominent microscopic feature was the perivascular proliferation formed especially by microglial elements. Special silver stains showed beyond doubt the microglial origin of these perivascular cells. In some instances scattered cells of a hematogenous nature were found in the perivascular spaces, mainly lymphocytes but occasionally also plasma cells. Musser and Hauser [1] found in their cases a general and diffuse parenchymal "round cell" infiltration, with many small hemorrhages in the vicinity of blood vessels and also hemorrhages filling the perivascular spaces.

Accompanying the reaction there was perivascular demyelinization with the presence of fat-containing glia cells.

An observation which is not a dominant feature but which may help to explain the pathogenesis of the lesions is the swelling or hyperplasia of the vascular lining endothelium. Ferraro and Scheffer [2] pointed to the vascular changes with the associated perivascular lesions and suggested that the causative agent is carried from the blood vessels to the surrounding tissues and is favored by an abnormal permeability of the protective wall and stasis of the venous system.

One should realize that there is no constant clinical picture of encephalitis in measles. Various authors stress first a diffuse involvement of the brain, with symptoms of headache, vomiting, dilatation of the pupils, muscular twitchings and convulsions. As the process continues, the diffuse features tend to be replaced by localized phenomena in one or more parts of the central nervous system. This, then, allows for classification into clinical types in which the encephalitis is accompanied by one of the following: hemiplegia and aphasia, paraplegia, a cerebellar syndrome or a spinal cord syndrome.

The cerebrospinal fluid presents no uniform picture. The fluid is usually clear and shows an increase in pressure, sometimes reaching as high as 400 mm. of water. The cell count may rise in some cases to 200, and these cells are almost all lymphocytes. The chloride content is usually not affected. The sugar content is uniformly normal or high. Neal and Applebaum [5] in most of their cases found a slight or moderate increase in protein, and occasionally a fluid would show an increase in number of cells without a corresponding increase in protein. More frequently they found that the protein was increased with or without a parallel increase in the number of cells. Smears and cultures of the fluid were always negative for micro-organisms.

Ford [6] stated that about 10 per cent of all patients die and that approximately 65 per cent of those who survive have residual symptoms, such as weakness, ataxia, mental deficiency, changes in personality, or epilepsy.

SUMMARY

A case of postmeasles encephalitis is reported. The most important changes were congestion of the blood vessels, perivascular microglial proliferation with some lymphocytic infiltration, and demyelinization with occasional phagocytic cells containing fat.

5. Neal, J., and Applebaum, E.: J. A. M. A. **88**:1552, 1927.
6. Ford, F. R.: Bull. Johns Hopkins Hosp. **43**:140, 1928.

ADENOCARCINOMA OF THE LARGE INTESTINE IN PRESUMABLY DIZYGOTIC TWIN BROTHERS

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Any departure from the normal structure or function in twins is of interest quite apart from the clinical value attaching to it. Every such case should be reported, and the report should state whether one or both twins are affected, and the age at which the defect appeared in the affected twin; it should also present all the available evidence on which the diagnosis of monozygosity or dizygosity is based. An accumulation of such records is of importance in determining the relative parts played by the genetic constitution of the patient and by extraneous forces in the development of the defect in question. For that reason we wish to report the following cases of tumors in twins.

REPORT OF CASES

The patients were brothers, A. M. and F. M. The father died at the age of 62, following an accident. The mother died at the age of 72, with pneumonia, following an intracapsular fracture of the hip. There were 3 sisters, 2 of whom were twins; these twins died at birth; the third sister is alive and well at 65, with no evidence of cancer. There was no history of cancer in the immediate family. A. M. and F. M. were twins presumably of the dizygotic type, there being little facial or bodily resemblance. One was tall and of medium build; the other was short and obese. They were most unlike in their dispositions. Further evidence of their probable dizygosity lies in the fact that their mother had had another pair of twins. According to both Greulich [1] and Guttmacher,[2] as well as others, the tendency to the production of dizygotic twins is hereditary but not that to the production of monozygotic twins. Thus there will often be a history of dizygotic twins not only in the mother's or the father's family but also in the immediate sibship. In the three pregnancies which Mrs. M. had, twins occurred twice.

CASE 1.—A. M. was aged 58½ and weighed 240 pounds (109 Kg.) when he first came for examination. He gave a history of increasing difficulty in obtaining a bowel movement over a period of three months, of cramplike pains in the left iliac region and of loss of appetite. Two years before examination he had been refused insurance because of hypertension. Roentgenograms taken in July 1933 showed an obstructive lesion at the proximal end of the sigmoid. An obstructive resection of the Rankin type was performed in August 1933, and the patient died seven weeks later.

From the Department of Anatomy, University of Western Ontario Medical School.

1. Greulich, W. W.: Am. J. Phys. Anthropol. **19**:391, 1934; J. A. M. A. **110**:559, 1938.

2. Guttmacher, A. F.: Am. J. Obst. & Gynec. **34**:76, 1937.

Pathologic Report.—The material received from the surgeon consisted of a piece of bowel 10 cm. long, with a firm mass at about the midpoint. On section, this appeared to be a friable papillary structure, projecting well into the lumen. At its base were cystic areas filled with gelatinous fluid. Similar cystic areas extended into the fatty tissue surrounding the intestine. The intestinal musculature was diminished and the wall fibrosed.

Microscopic Report.—The section was from the large bowel and showed atypical glandular tissue undermining the normal mucosa and invading the muscular coat. Cystic dilatations of the glands contained mucoid material. The cells and

Fig. 1 (case 1).—Low power field of a section at the margin of the tumor, showing normal mucosa at the upper left corner of the field and an atypical glandular mass, which has invaded the muscularis mucosa, occupying the center of the field. At the lower left are the large cystic areas filled with gelatinous material. At the right of the field the tumor extends to the edge of the section. Magnification, × 73.

the nuclei varied markedly in size; the nuclei were hyperchromatic, with prominent nucleoli. At places there was a heavy infiltration by polymorphonuclear cells. The cystic areas invaded the mesentery, although the adjacent lymph nodes were free from metastases. The diagnosis was adenocarcinoma of the bowel with extensions to the mesentery and with chronic inflammation.

Case 2.—In January 1935 F. M. consulted a surgeon. He was then 60. Rectal examination showed a new growth in the lower part of the ampulla. It appeared decidedly operable, but he delayed the operation for six weeks. An abdominoperineal

resection of the rectum was done in March 1935, and ten days later a second operation was performed for obstruction of the small intestine. He died the day of the second operation.

Pathologic Report.—The specimen received consisted of 14 cm. of rectum, displaying two raised tumor masses on its mucosal surface. The first was 1 cm. from the anal margin, was 3.5 cm. in diameter and was elevated about 7 mm. at its margins. The second mass was 5 mm. above the edge of the first, was 3 cm. in diameter and was elevated about 7 mm. above the mucosal surface. The tumor masses were slightly reddened as compared with the rest of the mucosa.

Fig. 2 (case 2).—Rectal tumor showing a fragment of normal mucosa at the upper right, with most of the field occupied by the carcinoma, which has invaded the muscular coat. Cystic dilatations of the glands filled with cellular material are to be seen. Magnification, × 73.

Microscopic Report.—The sections showed a transition from normal glandular tissue to a disorderly and irregular arrangement of glands. The normal mucosa was undermined and the muscularis invaded by the growth. The cells and nuclei varied in size, and the nuclei were hyperchromatic. Slight ulceration with chronic inflammatory reaction was present. The diagnosis was adenocarcinoma of the bowel with ulceration and chronic inflammation.

COMMENT

We have been unable to find in the literature any record of another case in which both twins were affected with cancer of the large intestine. It is quite possible that such cases have been reported in articles deal-

ing with rectal cancer without the title indicating that the condition existed in twins. Kranz [3] reported a pair of monozygotic male twins in whom cancer of the pylorus and of the rectum developed at 58 and 60, respectively. He also reported an instance of cancer of the rectum in one monozygotic twin brother at 69; the other twin was free from rectal cancer at the age of 74. Kranz reported a pair of dizygotic twin sisters in one of whom cancer of the rectum developed at 67; the other had cancer of the breast at 68. Still another case of Kranz's series was one of cancer of the rectum in a female twin at 50; the other twin, her brother, was living at 70, free from symptoms of cancer. In none of these pairs did both twins have cancer of the large intestine, although in the first pair mentioned by Kranz, both twins had cancers of the alimentary tract, one in the rectum, the other in the pylorus.

Because of the difference in site of the tumors reported in this paper, it is a question as to whether the case should be listed as an example of concordance of tumors in dizygotic twins, both showing adenocarcinoma of the large bowel, or whether it should be listed as an example of discordance of tumors in such twins, 1 having a tumor of the rectum, the other of the sigmoid. Developmentally, the sigmoid and the rectum have the same origin from the hindgut, and their separation into two parts of the digestive tract may merely be for purposes of anatomic classification. The incidence of cancer in the sigmoid is much less than that of cancer in the rectum, and this may indicate that tumors occurring at the two sites have a really different genetic basis. Or it may be considered that the genetic basis is the same in both cases and that external factors are responsible for the greater incidence of tumor in the rectum.

According to Ewing,[4] there is a distinct difference in the incidence of cancer in the sigmoid and in the rectum. He quotes Kaufmann's figures of 28 cancers of the sigmoid to 51 of the rectum in a series of 123 cases of intestinal cancer. This would be approximately 22.8 per cent of intestinal cancers in the sigmoid and 41.5 per cent in the rectum. In view of the fact that the sigmoid measures on the average a little more than three times as much as the rectum, an equal length of sigmoid should have 9 cancers instead of 28. This makes the incidence of cancer in the rectum really five and a half times as great as that in the sigmoid instead of the apparent one and four-fifth times derived from the comparison of 28 and 51 tumors, respectively. There seems, then, to be some actual difference in tissue susceptibility between the mucosa of the sigmoid and that of the rectum, in which case the tumors

3. Kranz, H., cited by Peyron, A., and Kobozieff, N: Bull. Assoc. franç. p. l'étude du cancer **26**:93, 1937.

4. Ewing, J.: Neoplastic Diseases, Philadelphia, W. B. Saunders Company, 1928, p. 710.

reported here would be discordant. If local conditions of blood supply, mobility of the bowel or degree of irritation from the progressively drying feces are factors enhancing the production of tumors, the increased incidence might be attributable to these local conditions modifying a similar genetic basis. These tumors would then be examples of concordance. Until more is known of the genetic, and especially of the local, factors responsible for the different incidence of tumors at various points along the digestive tract, the foregoing question cannot be settled.

Perhaps the relation of polyposis of the colon to cancer should be mentioned here. Polypi in the colon are said to be found in 75 per cent of the cases of cancer of the colon (Dukes [5]), and many of the cases of apparently hereditary cancer of the large intestine are thought to be cases of hereditary polyposis, on which a cancerous condition has been superimposed. There was no evidence of polypi in the segments of bowels removed at operation from these twin brothers.

It must be pointed out here that among some writers the misconception exists that a condition is apt to be hereditary only when found in both monozygotic twins and that its presence in both dizygotic twins is evidence that it has not a hereditary background. This is, of course, a false assumption. Just as brothers and sisters may have, and frequently do have, the same hereditary character, so both dizygotic twins, who are siblings as much as are two children born at different times to the same parents, may chance to inherit the same genetic factor for a particular character, thus resembling each other in that respect, although differing in their total heredity. Therefore, the fact that twins having the same type of tumor are dizygotic is no argument against that tumor having a genetic basis. The significance of tumors in monozygotic and dizygotic twins is discussed by one of the authors (M. T. M.) in a paper now in press.[6]

SUMMARY

Adenocarcinoma of the large intestine is reported as occurring in dizygotic twin brothers. The tumors differed in their anatomic location, one being in the sigmoid and the other in the rectum.

The twins were 58½ and 60 when the tumors developed. There was no history of cancer in the immediate family.

A survey of the literature does not reveal any other case of twins, whether monozygotic or dizygotic, in which both had cancer of the large intestine, although it is quite probable that such cases have been encountered.

5. Dukes, C.: Brit. J. Surg. **13**:720, 1925.

6. Macklin, M. T.: An Analysis of Cancer in Monozygous and Dizygous Twins, to be published.

General Review

ESTROGENS IN CARCINOGENESIS

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NEW HAVEN, CONN.

Simultaneously with the discovery and the subsequent chemical and biologic study of agents capable of producing neoplastic growths, it was discovered that certain substances in the body are capable of stimulating growth of the female genital tissues. The latter substances, which will be considered collectively as estrogens, are essential for normal proliferation and function of the female genital tissues. In fact, the experimental endocrinologist has been able to reproduce almost the entire sequence of uterine, vaginal and mammary changes in ovariectomized animals by proper application of ovarian estrogenic and luteal substances.[1]

Carcinogenic agents have been determined biologically by their activity in usually, but not always, inducing malignant growth of tissues to which they were applied directly. Estrogenic chemicals have been determined largely by their capacity to induce extensive but controlled or limited growth of the genital tissues, usually of the vagina or uterus. The cyclic nature of the sexual activity of female mammals is almost universal. During sexual maturity, under the influence of the ovary periods of proliferation are alternated with phases of regression. Interaction of the various hormones is presumably responsible for the cyclic growth and regression. Though it might be presumptive to assume an association between the factors stimulating normal growth—even excessively—and unrestricted or malignant growth, other evidence indicates such a relationship.

Chemists have demonstrated that among the phenanthrene derivatives carcinogenic activity depends to some extent on specific molecular configurations.[2] Hence, estrogens (those occurring in nature) could not be considered as resembling the carcinogens, though certain synthetic carcinogenic chemicals possess some estrogenic activity.[3]

From the Department of Anatomy, Yale University School of Medicine.

1. Allen, E.: Sex and Internal Secretions: A Survey of Recent Research, Baltimore, Williams & Wilkins Company, 1932.

2. Cook, J. W.; Haslewood, G. A. D.; Hewett, C. L.; Hieger, I.; Kennaway, E. L., and Mayneord, W. V.: Am. J. Cancer **29**:219, 1937. Cook, J. W., and Kennaway, E. L.: ibid. **33**:50, 1938. Shear, M. J.: ibid. **33**:499, 1938.

3. Cook, J. W.; Dodds, E. C.; Hewett, C. L., and Lawson, W.: Proc. Roy. Soc., London, s.B **114**:272, 1934. Cook, J. W.; Dodds, E. C., and Lawson, W.: ibid. **121**:133, 1936.

In view of the indefinite and meager knowledge of the fate of the carcinogens and estrogens in the body it appears possible that certain biologic changes at present attributed to them may be due in part to their metabolic derivatives.

INFLUENCE OF THE OVARY ON MAMMARY CARCINOGENESIS

Though ovaries had been removed from women [4] in attempts to alleviate cancer of the breast, the experimental demonstration of the influence of intrinsic hormonal factors on mammary carcinogenesis was first accomplished in mice.[5] The incidence of mammary tumors in nonbreeding animals varied in different lines but tended to be highest in strains of mice in which multiparous females acquired tumors most frequently.[5a] Mammary cancer developed only in female mice, more frequently in breeding than in virgin animals. Removal of the ovaries at the age of 3 to 5 months either completely inhibited or greatly delayed malignant changes. Ovariectomy at the age of 8½ to 10 months did not alter the incidence of cancer, while ovariectomy at the age of 5 to 7 months did not entirely prevent cancer.

Cori [6] likewise found that in mice ovariectomy at an early age (15 to 22 days) prevented the development of mammary tumors, while at a later age (2 to 5 months) it had less effect. Castration after the sixth month had little effect on the incidence of tumors. Using a large series of mice, Murray [7] reported the incidence of mammary tumors as follows: in normal breeding females, 80 per cent; in nonbreeding females, 11.5 per cent; in ovariectomized animals, 17 per cent, and in males bearing ovarian grafts, 7 per cent. Confirmation of the last point is found in the report of mammary carcinoma developing nine times and sarcoma twice in 16 castrated male mice bearing ovarian grafts.[8]

The sex-limited nature of mammary tumors in mice and the influence of ovarian grafts on the tendency for such tumors to develop in castrate males indicated the influence of the ovary. All investigators, however, did not obtain mammary tumors following transplantation of ovaries.[9] Male mice bearing intratesticular ovarian grafts did not acquire mammary tumors, though the grafts persisted and the mammary rudiments developed to a point at which they were equivalent to those

4. Cohen, 1909, cited by Loeb.[5c]

5. (*a*) Lathrop, A. E. C., and Loeb, L.: Proc. Soc. Exper. Biol. & Med. **9**:38, 1913; (*b*) J. Cancer Research **1**:1, 1916. (*c*) Loeb, L.: J. M. Research **40**:477, 1919.

6. Cori, C. F.: J. Exper. Med. **45**:983, 1927.

7. Murray, W. S.: J. Cancer Research **12**:18, 1928.

8. de Jongh, S. E., and Korteweg, R.: Acta brev. Neerland. **5**:126, 1935.

9. Lacassagne, A.: Compt. rend. Acad. d. sc. **195**:630, 1932; Loeb.[5c] Cori.[6]

of nonpregnant females.[10] Female mice in parabiosis with males did not acquire mammary tumors.[11] As ovarian grafts in males or the ovaries of females in parabiosis with males did not show development of corpora lutea, the failure to obtain mammary tumors may have resulted from the absence of luteal stimulation. On the other hand, it may be that a sufficiently high level of ovarian hormones is not attained under such conditions or that androgenic hormones may inhibit atypical growth.

Irradiation of mice with sufficiently large doses of roentgen rays to destroy the ovarian follicles to a great extent reduced the incidence of mammary tumors from 17.3 per cent in nonirradiated to 9.7 per cent in irradiated animals.[12] In 42 of the irradiated mice granulosa cell tumors developed, and 28.6 per cent of these mice showed mammary tumors, indicating the hormonal influence of the ovarian tumors on mammary carcinogenesis.

Ovarian Influences as Indicated by the Nature of the Sexual Cycle.—Though the estrous cycles of mice of different strains may vary, it usually has been impossible to associate them with the tendency toward development of tumors.[13] Suntzeff, Burns, Moskop and Loeb [14] followed the estrous cycles of mice of ten different inbred strains during a period of 32 days. They could not find that the duration of keratinization of the vaginal mucosa, the number of cycles or the degree of normality of the cycles as determined by the relationship of the estrous to the diestrous periods was associated with the incidence of tumors in the various strains (table 1). Dietary factors altered the cycles somewhat. Bonser [13b] noted that the application of estrogen in several ways to mice of two strains did not cause significant variation in the duration of cornification. Other investigators found that the estrous period (intervals of vaginal cornification) were longer and more regular in strains showing a high incidence of tumors.[15]

Later, after the study of a third strain of mice, Lacassagne [16] reported that his initial observations were only coincidental. More

10. Gardner, W. U.: Endocrinology **19**:656, 1935.

11. Murray, W. S.: Am. J. Cancer **30**:517, 1937.

12. Furth, J., and Butterworth, J. S.: Am. J. Cancer **28**:66, 1936.

13. (*a*) Loeb, L., and Genther, I. T.: Proc. Soc. Exper. Biol. & Med. **25**: 809, 1928. (*b*) Bonser, G. M.: J. Path. & Bact. **41**:33, 1935. (*c*) Suntzeff, V.; Burns, E. L.; Moskop, M., and Loeb, L.: Am. J. Cancer **26**:761, 1936. (*d*) Burns, E. L.; Moskop, M.; Suntzeff, V., and Loeb, L.: ibid. **26**:56, 1936.

14. Suntzeff and others.[13c] Burns and others.[13d]

15. Lacassagne, A.: Compt. rend. Soc. de biol. **115**:937, 1934. Harde, E.: ibid. **116**:999, 1934.

16. Lacassagne, A.: Am. J. Cancer **27**:217, 1936.

recently abnormal reproductive function has been noted in a strain of rats showing a high incidence of mammary tumors.[17]

As tumors developed in mice from tumor-susceptible strains, the estrous cycles soon became irregular and eventually stopped.[18] The genital tissues responded to minimal amounts of estrogens, but the ovaries eventually failed to respond to added hypophysial stimulation.

ESTROGENS AND MAMMARY CARCINOGENESIS

In order to facilitate presentation, the influence of estrogens on mammary carcinogenesis will now be discussed under three headings: (1) the influence of the strain of animals, (2) the amount and nature of the estrogens used in treatment and (3) the estrogenic chemical.

TABLE 1.—*Characteristics of the Estrous Cycles of Mice of Several Strains During a 32 Day Period of Observation*[13d]

Strain	Susceptibility to Mammary Cancer	Average Duration of Keratinization, Days	Cycles	Average Duration of Keratinization in Cycle, Days	Degree of Normality of Cycle, per Cent
NB	Medium low	15.8	5.6	3.0	62
A	High	15.3	5.7	2.7	53
OB	Low	15.0	4.6	3.2	50
C_{57}	Very low	13.2	4.6	2.9	14
CBA	Very low	11.9	6.7	1.8	62
D	High	11.1	4.6	2.4	25
M	Very low	9.6	5.5	1.8	50
C_3H	High	8.9	5.6	1.6	40
Y	Very low	6.9	3.7	1.8	33
N	Very low	6.2	5.5	1.1	77

Influence of Hereditary Factors, i. e., of the Strain of Mice.—Lacassagne[9] first obtained mammary cancers in mice following weekly injections of 0.03 mg. of ketoestrin benzoate (3-benzoate-17-keto-1,3, 5-estratriene). All of 3 male mice from a strain (R_3) in which 72 per cent of the untreated multiparous females showed spontaneous development of mammary adenocarcinoma gave rise to mammary tumors. In his initial paper Lacassagne raised the question whether the estrogen merely provided a morphologic stratum, the mammary glands, on which an intrinsic factor could act or whether it produced hyperplasia directly leading to cancer.

Mice have been a particularly desirable species for such studies, as geneticists have selected lines showing varying potentialities toward mammary carcinogenesis. These lines may be maintained by close

17. Bryan, W. R.; Klinck, G. H., and Wolf, J. M.: Am. J. Cancer **33**:370, 1938.

18. Allen, E.; Diddle, A. W.; Strong, L. C.; Burford, T. H., and Gardner, W. U.: Am. J. Cancer **25**:291, 1935.

inbreeding. The tendency to have mammary tumors is sex limited, however, males never or very rarely displaying such neoplasia. The mammary glands of males persist in a rudimentary condition throughout life.[19]

Lacassagne [20] immediately investigated the influence of estrogens on the incidence of mammary tumors in male and female mice from strains in which the multiparous females showed few spontaneous mammary tumors. The initial study indicated that the incidence of tumors in some strains of mice could be increased greatly by injection of estrogens. In one strain mammary tumors did not develop. In the high tumor strain (R_3) 11 of 12 male and 7 of 9 female mice after estrogen was injected presented mammary tumors.[21] Some of these tumors appeared at as early an age as 3½ months. A high rate of tumor development also occurred, however, in males and females from two other strains (17 and R_4) in which the incidence of spontaneous neoplasms in the untreated females was less than 2 per cent; 9 of 10 mice from these strains which survived for 8 months or more had mammary tumors at from 12 to 18 months of age. In these low tumor strains a much longer period of injection was required.

Male mice of strain A which received injections of 500 international units of estrogen weekly for periods of 162 to 362 days [22] presented 8 mammary tumors. In untreated multiparas of this strain the average age of appearance of spontaneous tumors was 307 days. Bonser [23] injected 300 to 500 international units of estrone (theelin; 3-hydroxy-17-keto-1,3,5-estratriene) weekly or painted mice with this substance dissolved in benzene. Mice of two strains (one high tumor and one low tumor) were used. Of 21 males of the high tumor strain which were treated 43 weeks or more, 3 showed development of mammary tumors. None of the 40 males from the low tumor strain living 43 weeks or more acquired tumors, though some lived for 80 weeks.

In 6 male mice of a high tumor strain receiving cutaneous applications of an estrogen dissolved in chloroform twice weekly mammary tumors developed, while no tumors appeared in mice of a mixed low tumor stock under similar treatment.[24] The numbers of mice treated were not stated.

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19. Gardner, W. U.; Diddle, A. W.; Allen, E., and Strong, L. C.: Anat. Rec. **60**:457, 1934.

20. Lacassagne, A.: Compt. rend. Soc. de biol. **114**:427, 1933.

21. Lacassagne, A.: Presse méd. **43**:233, 1935.

22. Gardner, W. U.; Smith, G. M.; Allen, E., and Strong, L. C.: Arch. Path. **21**:265, 1936.

23. Bonser, G. M.: J. Path. & Bact. **41**:217, 1935; **42**:169, 1936.

24. Cramer, W., and Horning, E. S.: (*a*) Lancet **1**:247 and (*b*) 1056, 1936.

Mammary tumors developed in 28 of 126 male and female mice (29 still living) which had been given ketoestrin benzoate or hydroxyestrin benzoate at the rate of 500 international units weekly for more than 125 days. The incidence of tumors was quite similar in treated animals from high tumor and low tumor strains.[25]

Using stock mice, Burrows [26] observed 3 mammary cancers among 130 mice following cutaneous applications of an estrogen dissolved in benzene. Perry and Ginzton [27] applied 1,2,5,6-dibenzanthracene alone and in combination with an estrogen (estrone, or theelin) cutaneously, in solution in benzene. Prior to the paintings some of the animals were ovariectomized. Table 2 gives the incidence of mammary tumors in mice living beyond the age of appearance of the first tumor. The incidence of spontaneous tumors in untreated females was considered to be very low.

TABLE 2.—*Incidence of Mammary Tumors in Ovariectomized Female Mice and in Female Mice Not Thus Operated on from a Low Tumor Stock Following Application of Carcinogens and Estrogens* [27]

	Animals	Mammary Cancers
Nonspayed females treated with 1,2,5,6-dibenzanthracene.....	25	0
Nonspayed females treated with 1,2,5,6-dibenzanthracene and theelin.	14	6 (43%)
Spayed females treated with 1,2,5,6,-dibenzanthracene.....	37	4 (11%)
Spayed females treated with 1,2,5.6-dibenzanthracene and theelin.....	15	1 (43%)

Mice from six strains in which the incidence of spontaneous tumors in multiparous females varied from nearly 0 to over 80 per cent were given variable amounts of an estrogen.[28] The mice of three strains showing a very low incidence of tumors (F, N and JK) did not show tumor development, though few survived for as long as 150 days. Among 111 mice of the other three strains (A and C_3H, which were high tumor stains, and CBA which was a low tumor strain) 48 mice presented mammary tumors, 4 showed lymphoid tumors, and 7 showed spindle cell sarcoma (table 3).

The injections were started at from 1 to 120 days of age. From 250 to 1,000 international units of hydroxyestrin benzoate or ketoestrin

25. Gardner, W. U.; Smith, G. M.; Strong, L. C., and Allen, E.: J. A. M. A. **107**:656, 1936.

26. Burrows, H.: Am. J. Cancer **24**:613, 1935.

27. Perry, I. H., and Ginzton, L. L.: Am. J. Cancer **29**:680, 1937.

28. Gardner, W. U., in Some Fundamental Aspects of the Cancer Problem: Symposium Sponsored by the Section on Medical Sciences of the American Association for the Advancement of Sciences, New York, Science Press, 1937, p. 67; Science, June 1937, supp. 4, p. 67.

benzoate was injected weekly, as a rule. Animals receiving smaller amounts of the less active estrogens showed few tumors.

Similar experiments with use of variable but usually smaller amounts of estrogen in aqueous or oily solution were conducted on six inbred strains of mice in another colony.[29] The numbers of males were not designated in all cases but nonbreeding females with a stated low tumor incidence constituted the greater part of the animals. The tumor incidence in multiparous females varied from 60.8 to 0 per cent, and that in

TABLE 3.—*Incidence of Mammary Tumors in Male and Female Mice of Three Strains Following Injection of Estrogens*[28]

Strain	Sex	Mice Treated	Av. Age of Mice at Time of Carcinogenesis, Days	Av. Age of Animals Dying Without Cancer, Days	Mice Showing					
					Mammary Cancers		Lymphoid Tumors		Sarcoma	
					Number	Per Cent	Number	Per Cent	Number	Per Cent
A	M	35	257	249	13	37.0	1	1.8	1	1.8
A	F	16	218	243	6	37.5	1	6.0	0	0
C3H	M	24	241	287	13	54.2	..	...	6	25.0
C3H	F	17	234	260	8	47.0	0	0	0	0
CBA	M	12	394	274	4	33.0	1	8.5	0	0
CBA	F	7	402	348	4	57.0	1	14.0	0	0
		111			48	43.0	4	3.6	7	6.3

TABLE 4.—*Incidence of Mammary Tumors in Estrogen-Treated Mice of Six Strains*[29]

Strain	Percental Incidence of Tumor in Nonbreeders Untreated	Percental Incidence in Multiparous Females	Mice Treated	Tumors in Treated Mice	Average Age at Carcinogenesis, Months	Percental Incidence of Tumors
C3H	2.5	60.8	6	5	6.9	83.3
D	6.4	5.15	12	6	10.1	50.0
A	4.5	43.1	37	12	13.3	32.4
NB	13.9	31.9	25	5	16.4	20.0
OB	0	9.1	10	0	0	0
C57	0	0	12	0	0	0

the treated mice from 83.3 to 0 per cent (table 4). Fifty-three per cent of the 13 males and 29 per cent of the 27 females, receiving 50 to 100 rat units of estrogen in oil weekly, showed tumors.

Mice of the CBA strain have not shown spontaneous development of mammary tumors in some laboratories.[30] With weekly injection of 500 international units of estrone benzoate (ketohydroxybenzoate) in olive

29. Suntzeff, V.; Burns, E. L.; Moskop, M., and Loeb, L.: Am. J. Cancer **27**: 229, 1936.

30. Bonser, G. M.; Stickland, L. H., and Connal, K. I.: J. Path. & Bact. **45**: 709, 1937.

oil into 22 females and cutaneous application of estrone and injection of the lactogenic substance from the anterior lobe of the pituitary (prolactin) to 10 other mice, mammary tumors appeared in 5 mice after 55 to 103 weeks of treatment.

The observations on mice with known incidences of tumor appearance indicate that intrinsic factors other than estrogens play a significant role in mammary carcinogenesis. In some strains of mice, however, the influence of these intrinsic factors may be entirely or largely dominated by the effects of estrogens administered for long periods. In other strains, characterized by very low incidence of spontaneous carcinogenesis, few mammary tumors have been obtained by administration of estrogens.

Response to Various Estrogenic Chemicals.—Various estrogens have been injected into male mice for the purpose of comparing their relative "carcinogenic" effects on mammary tissues. Mammary tumors developed in male mice receiving the benzoates of equilin (3-hydroxy-17-keto-1,3,5,7-estratraene), equilenin (3-hydroxy-17-keto-1,3,5,6,8-estrapentaene) and estrone [31] (table 5).

TABLE 5.—*Incidence of Mammary Tumors in Mice of the High Tumor R_3 Strain Following Injection of Three Different Estrogens* [31b]

Estrogenic Chemical	Mice Treated	Mice Showing Tumors	Av. Age at Development of Tumor, Days
Estrone benzoate	3 males, 1 female	2 males	214
Equilin benzoate	4 males, 1 female	4 males, 1 female	199
Equilenin benzoate	4 males, 1 female	3 males, 1 female	338

Four or 5 mice of the R_3 strain, in which the incidence of mammary cancer is 72 per cent, were given weekly injections of 0.05 cc. of an oily solution (1 part in 1,000) of estrone benzoate, equilin benzoate or equilenin benzoate.[31b] In the mice receiving equilin benzoate the tumor incidence was 100 per cent; in the mice receiving equilenin benzoate, if a mouse that died at twenty-one days is excluded, the incidence was also 100 per cent. In the estrone-treated animals there was a high mortality, which resulted in a low percentage of animals showing tumors. Lacassagne concluded that equilin was as carcinogenic as estrone and in view of the very late appearance of tumors in the mice receiving equilenin, was much more active than the latter. Variations in the resulting mammary hyperplasia and morphologic changes in the uterus, in the pituitary and in the development of the mice were also studied. Estrone produced the most profound changes, then equilin and equilenin.

31. Lacassagne, A.: (*a*) Compt. rend. Soc. de biol. **122**:183, 1936; (*b*) Am. J. Cancer **28**:735, 1936.

Mammary tumors were observed in male mice which had received estrone (theelin), estriol (theelol), equilin, equilin benzoate, hydroxyestrin benzoate and ketoestrin benzoate.[28] Though few of the data were presented, it was stated that the carcinogenic activity tended, within limits, to be related to the amount of estrogen in physiologic units rather than to the kind of estrogen. Twenty-one mammary and 4 lymphoid tumors were observed among 73 mice given 0.1 mg. of equilin (332 mouse units), equilin benzoate (500 mouse units) or equilenin benzoate weekly[32] (table 6). The assays of the two chemicals just mentioned on mice were considerably lower than the usual assays of these substances in oil solution.

Burrows[33] compared the action of seven estrogenic chemicals on the mammary glands and other tissues of mice. Estrone, 9,10-dihydroxy-9,10-di-n-propyl-9, 10-dehydro-1,2,5,6-dibenzanthracene, equilenin, the

TABLE 6.—*Mammary Tumors in Mice of the High Tumor C3H and A Strains Following Injection of Various Estrogens*[32]

Strain	Sex	Estrogenic Chemical*	Mice Treated	Mice Showing Mammary Tumors	Mice Showing Lymphoid Tumors
A	F	EB	4	1	..
A	M	EB	10	1	..
A	F	E	2	..	..
A	M	E	12	..	..
C3H	F	EB	2	1	..
C3H	M	EB	14	4	4
C3H	F	E	5	5	..
C3H	M	E	11	2	..
C3H	F	ENB	6	4	..
C3H	M	ENB	7	3	..
			73	21	4

* EB is equilin benzoate; E, equilin, and ENB, equilenin benzoate.

methyl ester of estrone, equilin, estradiol (3,17-dihydroxy-1,3,5,-estratriene) and estriol were applied in benzene or alcoholic solution to the skin. Mammary cancer developed in 3 animals receiving estrone.

Mammary adenocarcinoma developed in male mice receiving all the various normally occurring estrogens studied. At the present time the influence of the various chemicals on the formation of tumors appears to be proportional to their physiologic activity.

Amount of Estrogen Administered.—With minimal amounts of estrogen the tumors tended to be few.[29] If the mice are grouped without consideration of strain, 15, 14.71 and 62.5 per cent presented tumor development following daily injection of ⅓ to 1, ½ to 10 and 20 rat units of estrogen in aqueous solution. When 50 rat units was given twice weekly, tumors developed in 20 per cent of the mice.

32. Gardner, W. U.; Smith, G. M.; Allen, E., and Strong, L. C.: To be published.

33. Burrows, H.: Brit. J. Surg. **23**:191, 1935; J. Path. & Bact. **42**:161, 1936.

Few mice showed tumors when very small amounts of estrogen were given, though insufficient data were available to draw definite conclusions.[28] The incidence of mammary tumors in male mice receiving 100 to 250 international units of hydroxyestrin benzoate weekly tended to be much lower than that in mice receiving 500 international units. The incidence of tumors in mice receiving 1,000 units weekly tended to be lower, owing in part to the inability of mice to tolerate such treatment over long periods.

INFLUENCE OF OTHER "SEX HORMONES" ON MAMMARY CARCINOGENESIS

Testosterone.—The mammary glands of rats receiving 200 micrograms of testosterone (3-keto-17-hydroxy-4-androstene) daily will undergo considerable hypertrophy.[34] Similar observations have been made by Astwood, Geschickter and Rausch [35] and by Geschickter and Astwood.[36] Little mammary development, however, occurs in mice receiving 1.25 to 2.5 mg. of testosterone propionate weekly.[37] Mammary tumors have not been observed in mice so treated. Burrows [38] observed that testosterone inhibited the development of the nipples in female mice.

Three mice of the R_3 strain survived more than 120 days when given 100 micrograms of estrone and 500 to 1,000 micrograms of testosterone acetate on alternate weeks. In 2 of these mammary adenocarcinoma developed.[39] Mammary tumors developed in both of 2 females of this high tumor strain which had received 500 to 1,000 micrograms of testosterone acetate weekly, but no tumors developed in 5 male mice so treated. Mammary tumors appeared in mice of the high tumor C_3H strain which had received weekly 500 international units of hydroxyestrin benzoate and 1.25 to 2.5 mg. of testosterone propionate.[40] The number of animals and of completed experiments is yet too small to permit determination of the influence of testosterone on the incidence of tumor.

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34. McEwen, C. S.; Selye, H., and Collip, J. B.: Proc. Soc. Exper. Biol. & Med. **35**:56, 1936. Selye, H.; McEwen, C. S., and Collip, J. B.: ibid. **34**:201, 1936.

35. Astwood, E. B.; Geschickter, C. F., and Rausch, E. O.: Am. J. Anat. **61**: 373, 1937.

36. Geschickter, C. F., and Astwood, E. B., in Some Fundamental Aspects of the Cancer Problem: Symposium Sponsored by the Section on Medical Sciences of the American Association for the Advancement of Sciences, New York, Science Press, 1937, p. 76; Science, June 1937, supp. 4, p. 76.

37. Gardner, W. U.: Unpublished data.

38. Burrows, H.: J. Path. & Bact. **44**:699, 1937.

39. Lacassagne, A.: Compt. rend. Soc. de biol. **126**:385, 1937.

40. Gardner, W. U.: Unpublished data.

Though testosterone may have a slight effect on the proliferation of mammary tissue, it does not appear to inhibit the development of spontaneous tumors in females or of tumors arising in males receiving estrogens.

Progesterone.—Progesterone was given along with an estrogen for seventy days to a mouse in which a mammary tumor developed later, after the injection of progesterone had been discontinued.[38] Progesterone and estrone were given to mice of the R_3 strain on alternate weeks (50 to 100 micrograms of estrone and 200 micrograms of progesterone) or simultaneously (50 micrograms of estrogen and 100 micrograms of progesterone); in 8 of 10 mice which survived over 120 days mammary adenocarcinoma developed at 123 to 194 days.[39] Mammary tumors developed in 3 of 6 male mice of the C_3H strain receiving 200 international units of hydroxyestrin benzoate and 400 micrograms of progesterone weekly.[40] Mammary tumors did not develop in mice receiving progesterone alone.

Progesterone alone in very large doses (50 to 100 micrograms daily) stimulated slight mammary hypertrophy in male mice,[41] but doses of 4 mg. daily in adult rats failed to induce this change.[42]

These experiments indicate that progesterone alone does not markedly stimulate mammary tissue and when given with estrogens has little if any effect on the development of mammary tumors.

INFLUENCE OF "SEX HORMONES" ON NORMAL AND "PRECANCEROUS" BREAST CHANGES

The rudimentary or small breasts of male or ovariectomized immature female mice develop rapidly after injection of estrogens.[43] In hypophysectomized mice, however, these rudiments did not respond.[44] The influence of ovarian hormones on the breast has been recently reviewed.[45] It may be stated here that the administration of estrogens

41. Gardner, W. U., and Hill, R. T.: Proc. Soc. Exper. Biol. & Med. **34**: 718, 1936.

42. Selye, H.; Browne, J. S. L., and Collip, J. B.: Proc. Soc. Exper. Biol. & Med. **34**:472, 1936.

43. (*a*) Turner, C. W.; Frank, A. H.; Gardner, W. U.; Schultz, A. B., and Gomez, E. T.: Anat. Rec. **53**:227, 1932. (*b*) Turner, C. W., and Gomez, E. T.: The Normal Development of the Mammary Gland of the Male and Female Albino Mouse, Research Bulletin 182, University of Missouri, College of Agriculture, Agricultural Experiment Station, 1933; Development of the Mammary Gland: I. The Male and Female Albino Mouse; II. The Male and Female Guinea Pig, Research Bulletin 206, ibid., 1934. (*c*) Gardner and others.[19]

44. Gomez, E. T.; Turner, C. W.; Gardner, W. U., and Hill, R. T.: Proc. Soc. Exper. Biol. & Med. **36**:287, 1937.

45. Turner, C. W.: The Mammary Glands, in Allen,[1] chap. 12. Nelson, W. O.: Physiol. Rev. **16**:488, 1936.

to mice with intact pituitaries resulted in proliferation of the glandular ducts. The simultaneous administration of progesterone resulted at times in some alveolar proliferation.[43b]

Goormaghtigh and Amerlinck [46] were the first to describe changes occurring in the mammary tissue of mice receiving estrogens over long periods. Similar changes were observed by Lacassagne and compared to the clinical findings in Shimmelbusch's disease.[47] The first change noted was general or irregular cystic distention of the ducts with a colostrum-like fluid. Without evidence of inflammatory reaction, localized proliferation of the epithelium occurred in some of the hyperplastic breast tissues. In such tissues carcinomatous areas developed.[21] The "precancerous" changes in the breasts occurred earlier in male mice of high tumor strains than in those of low tumor strains.

Observations were made on the mammary glands of 171 male mice, of which 53 were controls, 23 untreated and 30 treated by cutaneous applications of benzene.[33] To other mice the estrogens were administered cutaneously in benzene solution. The mammary tissue was located by using the inguinal lymph node; consequently the rudiments were found in the controls only occasionally (17.2 and 21 per cent). Mammary tissue was found in 63.9 and 46.7 per cent of the mice in groups which received estrone (theelin) and other estrogens dissolved in benzene. The changes in the breast tissue were described as (1) gynecomastia or hypertrophy of the breast tissues, (2) dilatation of the ducts, (3) hyperplasia of the epithelium, (4) leukocytic infiltration, (5) increase in periductal fibrous tissue and (6) benign adenoma.

The proliferation of the mammary ducts occurred in mice receiving estrone, 9,10-dehydroxy-9,10-d-n-propyl-9,10-dehydro-1,2,5,6-dibenzanthracene, equilenin, the methyl ester of estrone, equilin, estradiol and estriol.[33] Though some alveolar proliferation was evident in mice of all groups, it was greatest in those receiving estradiol and equilin. The equilin and estradiol solutions were ten times as concentrated as the estrone solutions, and it seems probable that the variations in response might have been due to quantitative differences in the doses.

The breast tissues of two strains of mice were studied after injection of estrone in olive oil or cutaneous application of estrone in benzene.[23b] The glands of the fourth or fifth inguinal pair were located by the inguinal lymph node. Localized alveolar formation occurred in several mice of the high tumor strain, while less active alveolar and more generalized proliferation was apparent in the low tumor strain. Intraductal hyperplasia, anaplasia and neoplasia were never observed. The ducts were particularly quiescent in the strain in which carcinoma

46. Goormaghtigh, M., and Amerlinck, A.: Compt. rend. Soc. de biol. **103**: 527, 1930.

47. Cheatle, L.: Brit. J. Surg. **22**:710, 1935.

developed (Bonser [13b]) ". . . localized alveolar proliferation is of more importance than cystic duct distention in the subsequent development of cancer in these mice."

Cystic distention and glandular hyperplasia were more frequently observed in mice which had received an extract of the corpus luteum, estrogen or pituitary substances alone or in combinations.[48] The extract of corpus luteum produced abnormal development of the breast in the highest percentage of mice.

A procedure whereby the entire mammary system could be examined [19] was used in a study of the effects of large amounts of estrogen.[49] All of the mammary rudiments did not respond, some persisting as small rudimentary ducts. In the same mice, other mammary glands developed during the same period but were uniformly smaller than the glands of mice which had received smaller amounts of estrone and were therefore designated as "stunted glands." Localized areas of variable size showed extensive alveolar development. Some of these local nodules were adenomatous in character. In the adenomatous areas early malignant changes were observed. Leukocytic infiltration and fibrosis of the stroma were observed occasionally. The ducts usually were distended with secretion, which in the fixed material tended to form concretion-like masses.

Mammary changes of the type described were found in mice of two high tumor strains and one low tumor strain but were not observed in mice of the very low tumor strains.[28] In fact, in one low tumor strain the mammary rudiments responded very slightly if at all to the larger amounts of estrogen (500 international units) whereas they developed extensively when smaller amounts were given. By increasing the amount of estrogen given the ultimate size of the glands could be further restricted.

Most of the mammary changes occurring in male mice receiving estrogens in large amounts, with the exception of the stunting, may be observed likewise in the glands of old female mice of the same strains.[50] The tendency toward localized alveolar proliferation and adenomatous change was particularly common in the high tumor strains, though in these cystic distention of the ducts was less common. Such hyperplastic areas were extremely rare in old females of the tumor-resistant strains.

The nature of the abnormal mammary growth described by different investigators in male mice chronically treated with large amounts of estrogen has been quite uniform. One group of investigators, however,

48. Wiesner, C.: Arch. f. Gynäk. **154**:548, 1933; **156**:534, 1934.

49. Gardner, W. U.; Smith, G. M., and Strong, L. C.: Proc. Soc. Exper. Biol. & Med. **33**:148, 1935.

50. Gardner, W. U.; Strong, L. C., and Smith, G. M.: Am. J. Cancer, to be published.

tended to stress the ductal metaplasia and intraductal papillomatous growths as the major precancerous condition whereas the other stressed the localized alveolar proliferation and adenomatous growth. The absence or rarity of certain of these abnormal mammary changes, particularly the hyperplastic nodules, in mice of the very low tumor strains indicates that those changes may be related to the changes leading to malignancy.

The mammary glands of most species respond to injections of estrogen. The glands of comparatively few species have been studied after long periods of estrogen stimulation. In rabbits when small amounts of estrogen (20 rat units daily) were given for 120 days involution of the breast occurred during the later stages.[51] Macdonald[52] made similar observations, though he stressed the hyperplastic and cystic state of the ducts during the earlier stages of the treatment. Sixty to 90 days after the administration of estrogen was started lactation began.[53]

In the breasts of immature ovariectomized or young male monkeys given estrogenic treatment there developed first a branching network of ducts and then lobules of alveoli. The presence of many mitotic figures in the breast removed from an animal twenty-one and a half weeks after treatment started, with administration of 136,000 international units of hydroxyestrin benzoate, indicated a prolonged influence of the estrogen.[54] The proliferation of mammary tissue persisted long after growth in the uterus had stopped.

The injection of small amounts of estrone (16 to 64 rat units daily) for six weeks led to proliferation of the mammary ducts and nipples in rhesus monkeys. The ductal epithelium was hyperplastic and poorly organized, and the ducts were distended with secretion. This condition was compared to clinical gynecomastia.[55]

Six castrate female rats which had received injections of 30 micrograms of estrone daily for 331 days showed multiple mammary cysts and an adenofibroma.[56] Large doses of estrogen modified the structure of the mammary glands of rats, resulting in stunting of the duct tree and in cyst formation.[57]

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51. Turner, C. W., and Frank, A. H.: The Effect of the Ovarian Hormones Theelin and Corporin upon the Growth of the Mammary Gland of the Rabbit, Research Bulletin 174, University of Missouri, College of Agricultural Experiment Station, 1932.

52. Macdonald, I. G.: Surg., Gynec. & Obst. **63**:138, 1936.

53. Frazier, C. N., and Mu, J. N.: Proc. Soc. Exper. Biol. & Med. **32**:997, 1935.

54. Gardner, W. U., and Van Wagenen, G.: Endocrinology **22**:164, 1938.

55. Geschickter, C. F.; Lewis, D., and Hartman, C. G.: Am. J. Cancer **21**: 828, 1934.

56. McEwen, C. S.; Selye, H., and Collip, J. B.: Lancet **1**:995, 1936.

57. Herold, L., and Effkemann, G.: Arch. f. Gynäk. **163**:85 and 94, 1936. Astwood, E. B., and Geschickter, C. F.: Arch. Surg. **36**:372, 1938.

UTERINE RESPONSES TO "SEX HORMONES"

Malignant Growths.—An epithelioma of the uterus was observed in a mouse of a low tumor strain which had received estrone and pituitary extract.[58] One uterine horn of this mouse showed pyometra.

Three mice of a group of 27 which received estrone (125 rat units and 62.5 rat units approximately) and 0.1 cc. of a 0.3 per cent solution of 1,2,5,6-dibenzanthracene weekly in solution in benzene showed cervical carcinoma at 6 to 10 months after treatment started.[59] Two of the mice had pyometra and breast tumors. The largest tumor had invaded the surrounding pelvic tissues.[27] Uterine or cervical tumors were not observed in mice receiving 1,2,5,6-dibenzanthracene alone.

Loeb, Burns, Suntzeff and Moskop[60] observed a "carcinoma-like" proliferation of the cervix of a mouse of the low tumor "Old Buffalo" strain. This mouse had been treated for over 24 months. Ten rat units of theelol had been administered daily for eighteen months and 30 rat units of theelin for the remaining 6 months. The tumor had invaded the muscularis and subserosal layers. It consisted of strands or cords of squamous epithelium.[61] The same investigators studied the vaginas and cervices of 128 adult untreated mice and of 235 mice which had received estrogens alone or in combination with luteal or anterior pituitary substances. These mice were from seven different strains. Epithelial irregularities of the upper part of the vagina and cervix were noted with increasing age in both treated and untreated groups but were particularly pronounced in the former. In 26 of the treated animals precancerous or cancer-like lesions (proliferations graded 3 or 4) were found, the animal referred to in the first statement in this paragraph showing the most extensive change. The atypical epithelial changes occurred irrespective of the tendency to have mammary tumors or of the strain of mice.

Similar epithelial overgrowths appeared in 5 mice of three different strains which had received 500 international units of ketoestrin or hydroxyestrin benzoate.[28] As these overgrowths, some of them infiltrating the muscularis and subserosa, were not observed in the living animals, they were not studied after the cessation of estrogenic injections and were not grafted. It cannot be stated that they would have grown in the absence of further stimulation. Later a large cervical tumor among 19 which had been found up to that time was successfully grafted into

58. Lacassagne, A.: Compt. rend. Soc. de biol. **121**:697, 1936.

59. Perry, I. H.: Proc. Soc. Exper. Biol. & Med. **35**:325, 1936.

60. Loeb, L.; Burns, E. L.; Suntzeff, V., and Moskop, M.: Proc. Soc. Exper. Biol. & Med. **35**:320, 1936.

61. Suntzeff, V.; Burns, E. L.; Moskop, M., and Loeb, L.: Am. J. Cancer **32**:256, 1938.

other mice of the same strain.[62] The mouse yielding this tumor had received 10,500 international units of hydroxyestrin benzoate in 319 days. The carcinoma had invaded most of the pelvic tissues, and metastatic growths were found in the lumbar lymph nodes. Though this was the only tumor which proved to be malignant, it is probable that the other 18 represented earlier stages of the same condition. The earlier development of mammary tumors probably lowered the incidence of uterine tumors.[62] It was necessary to remove 2 mammary tumors prior to the appearance of the aforementioned large cervical tumor.

The incidence of uterine tumors in untreated mice was extremely low, and most of these originated in the muscular or stromal tissues.[63] In view of the low incidence of uterine carcinoma in untreated mice the development of this type of tumor in animals receiving estrogens is of particular significance. The uterus, like the mammary glands, responds to estrogens, eventually giving origin to cancer, though in the case of the uterine tumor evidence of intrinsic or hereditary factors is lacking. The malignant changes are preceded by extensive proliferation in both instances—"estrogenic hormones evidently induce the development of cancer in those organs in which they call forth long-continued or often-repeated growth processes." [64]

Physiologic and Abnormal Hyperplasias.—(*a*) Deciduoma, endometrial mole and implantation sites. The influence of ovarian endocrine activity, particularly the production of corpus luteum, on atypical uterine responses was first indicated by Loeb.[65] Traumatization of the endometrium of the guinea pig's uterus during the early part of the luteal phase of the cycle led to the formation of decidual overgrowths called placentoma or deciduoma. These growths were transitory. Such limited uterine responses following the administration of pure "hormones" have been studied extensively in several species (see reviews [66]). It is also probable that pituitary hormones may affect the development of deciduoma to some extent.[67] Another abnormal but limited uterine response (the endometrial mole) has been observed in rats in which the uteri have been traumatized in certain physiologic states.[68]

62. Gardner, W. U.; Allen, E.; Smith, G. M., and Strong, L. C.: J. A. M. A. **110**:1182, 1938.

63. Selye, M.; Holmes, H. F., and Wells, H. G.: J. Cancer Research **8**:96, 1924.

64. Loeb, L.: Scient. Monthly **47**:51, 1938.

65. Loeb, L.: J. A. M. A. **50**:1897, 1908.

66. (*a*) Hisaw, F. L., in Allen,[1] chap. 11. (*b*) Selye, H., and McKeown, R.: Proc. Roy. Soc., London, s.B **119**:1, 1935.

67. Evans, H. M.; Simpson, M. E., and Turpeinen, K.: Anat. Rec. (supp.) **70**:26, 1938.

68. Selye, H.; Harlowe, C., and McKeown, T.: Proc. Soc. Exper. Biol. & Med. **32**:1253, 1935. Selye and McKeown.[66b]

The uterus of the rhesus monkey also responds to traumatic stimuli when under the influence of the luteal hormone.[69] This response is similar to but more extensive than that occurring in monkeys at the time of implantation.[70]

(*b*) Cystic hyperplasia. Cystic endometrial hyperplasia has been discussed extensively in the medical literature. More recently it has been studied in experimental animals. Cystic endometrial hyperplasia developed following the injection of 10 to 50 rat units of estrogen daily into rats and guinea pigs.[71] Similar hyperplasia has been observed in mice,[72] guinea pigs,[73] rabbits[74] and monkeys.[75] Comparatively large amounts of estrogen were required in most of these experiments, and the duration of treatment was long in most cases. In some species, particularly in rabbits and guinea pigs, the hyperplasia was associated with endometrial hemorrhage. Zondek[74b] obtained a series of changes, somewhat progressive in their appearance, namely, (1) hyperemia, (2) extravascular hemorrhage, (3) glandular cystic hyperplasia and (4) aseptic suppuration and necrosis. Hyperplasia in monkeys was of less marked extent; in fact, it was not observed by several investigators.[76]

Cystic endometrial hyperplasia, though apparently not occurring frequently in untreated experimental animals, may follow many procedures involving ovarian manipulation. In guinea pigs partial ovariectomy (complete removal of one ovary and partial removal of the other[77]) and roentgen treatment of the ovaries[78] have resulted in such abnormal overgrowth of the uterus. The grafting of testes of newborn mice into their sisters altered the ovarian physiology of the latter so that cystic

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69. Hisaw, F. L.; Greep, R. O., and Fevold, H. L.: Am. J. Anat. **61**:483, 1936.

70. Wislocki, G. B., and Streeter, G. L.: On the Placentation of the Macaque (Macaca Mulatta) from the Time of Implantation Until the Formation of the Definitive Placenta, Publication 496, Carnegie Institution of Washington, 1938.

71. Burch, J. C.; Williams, W. L., and Cunningham, R. S.: Surg., Gynec. & Obst. **53**:338, 1931. Wolfe, J. M.; Campbell, M., and Burch, J. C.: Proc. Soc. Exper. Biol. & Med. **29**:1263, 1931.

72. (*a*) Parkes, A. S.: Lancet **1**:485, 1937. (*b*) Gardner, W. U.; Allen, E., and Strong, L. C.: Anat. Rec. (supp.) **64**:17, 1936.

73. (*a*) Nelson, W. O.: Anat. Rec. **68**:99, 1937. (*b*) Dessau, F.: Arch. internat. de pharmacodyn. et de thérap. **55**:402, 1937; (*c*) **58**:344, 1938.

74. (*a*) Lacassagne, A.: Compt. rend. Soc. de biol. **120**:685, 1935. (*b*) Zondek, B.: J. Exper. Med. **63**:789, 1936. (*c*) Pierson, H.: Ztschr. f. Krebsforsch. **41**:103, 1934; **45**:1, 1936.

75. Zuckerman, S., and Morse, A. H.: Surg., Gynec. & Obst. **61**:15, 1935. Zuckerman, S.: J. Obst. & Gynaec. Brit. Emp. **44**:494, 1937.

76. (*a*) Engle, E. T., and Smith, P. E.: Anat. Rec. **61**:471, 1935. (*b*) Gardner, W. U., and Van Wegenen, G.: Anat. Rec. (supp.) **70**:29, 1938. Hisaw and others.[69]

77. Lipschütz, A.: Gynéc. et obst. **36**:408 and 481, 1937.

78. Schmidt, I. G.: Anat. Rec. (supp.) **70**:69, 1938.

uteri were obtained.[79] These experimental procedures, though probably not effecting an increase in the production of ovarian hormones, may alter factors responsible for the rhythm of the regressive and hyperplastic phases characterizing the normal cycle.

Progesterone, the active substance in corpus luteum, prevented the pronounced hyperplasia when administered with estrogens.[80] The changes were less readily attained in rats not operated on than in castrate rats given estrogens, presumably because of the absence of corpora lutea.[81]

Cystic endometrial hyperplasia appears to be a sequence of chronic estrogen stimulation uninterrupted, as during the normal cycle, by the degeneration or luteinization of the ovarian follicles. The continuity of treatment rather than the amount of the estrogenic substance above a certain minimum seems most essential.

(*c*) Metaplasia of the uterine cervix and body. The morphologic similarity of the cervix of the monkey to that of the human being led to investigations of the effect of estrogens on abnormal growths of the former species. Epithelial metaplasia of the cervical glands occurred in monkeys which had received 50 to 100 rat units of estrogen daily for 16 to 90 days.[82] Beginning at the distal portion of the glands, areas of epithelial metaplasia and stratification appeared. Histologically, precancerous areas developed when the cervix was subjected to trauma at the same time. Later other investigators observed the epithelial changes in the cervixes of monkeys receiving estrogens in relatively large amounts over variable periods.[83] It is generally agreed that the growths were metaplastic and nonmalignant. The tissues returned to their original state after the cessation of injections or following the administration of adequate amounts of progesterone. The simultaneous injection of progesterone prevented these abnormal changes.[83a]

Metaplasia of the cervical glands occurred also in guinea pigs following injection of estrogens over long periods.[80] In rats and mice the cervical canals are normally lined by a stratified squamous epithelium and contain no mucus-secreting glands. Metaplasia has not been described in them though the squamous epithelium may extend into the uterine horns. In mice which have received estrogens for long periods, the epithelium of the cervical canal, vaginal fornices and upper

79. Pfeiffer, C. A.: Anat. Rec. (supp.) **70**:62, 1938.

80. Nelson.[73a] Dessau.[73b]

81. Migliavacca, A.: Arch. f. Gynäk. **159**:172, 1935; **162**:595, 1936.

82. Overholser, M. D., and Allen, E.: Proc. Soc. Exper. Biol. & Med. **30**: 1322, 1933; Surg., Gynec. & Obst. **60**:129, 1935.

83. (*a*) Hisaw, F. L., and Lendrum, F. C.: Endocrinology **20**:228, 1936. (*b*) Migliavacca, A.: Arch. f. Gynäk. **164**:463, 1937. (*c*) Zuckerman, S.: Lancet **1**:435, 1937. (*d*) Engle and Smith.[76a]

part of the vagina may come to have a thickened, noncornified layer over a very irregular basement membrane.[61] In these regions small invasive growths have occasionally been observed.

Epithelial metaplasia also occurred in the uterine cornua of rats, mice and guinea pigs which had received estrogens. The intraperitoneal administration of 30 to 60 micrograms of estrone daily for 10 weeks resulted in epithelial metaplasia in 4 of 8 rats.[84] Similar changes occurred in 4 days following intrauterine injections.[85] Five of 6 rats given 30 micrograms of estrone subcutaneously daily showed epithelial metaplasia and hypertrophic fibrosis of the lower layers of the mucosa. Observations of the same nature have been made by other investigators.[86] The changes were found often in ovariectomized animals.[81] In some cases they were accompanied by septic inflammatory reactions as well as by hypertrophic fibrosis and stromal hyalinization.

Metaplastic changes were found in the uteri of mice, particularly when the injections were started in very young animals.[72b] In older animals receiving large amounts of estrogens metaplasia occurred less regularly and was associated with cystic glandular hyperplasia or septic inflammation. In all these cases the stroma had undergone fibrosis or hyalinization. Epithelial metaplasia did not occur in uterine horns ligated near the cervix or in uterine horns grafted subcutaneously, although these persisted in a cystic state throughout the period of treatment.[87]

Pyometra frequently developed in mice receiving estrogens in large amounts.[88] Death frequently occurred after a few weeks from peritoneal involvement. Bacterial invasion was apparently responsible for the suppurative uterine inflammation, as transplanted uteri did not show pyometra. Bacteria were identified in the uteri of most mice within from 1 to 2 weeks after the subcutaneous injection of 500 international units of hydroxyestrin benzoate.[89] Septic uterine inflammation developed only after very extended treatment in rats [86a] and did not appear in rabbits.[74b]

Epithelial metaplasia of the uterine fundus has not been described in monkeys. After a period of epithelial proliferation, largely completed

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84. Selye, H.; Thomson, D. L., and Collip, J. B.: Nature, London **135**:65, 1935. McEwen and others.[56]

85. McEwen, C. S.: Am. J. Cancer **27**:91, 1936.

86. (*a*) Kaufmann, C., and Steinkamm, E.: Arch. f. Gynäk. **162**:553, 1936. (*b*) Gumbrecht, P.: ibid. **160**:525, 1936. (*c*) Herold, L., and Effkemann, G.: Zentralbl. f. Gynäk. **61**:115, 1937. (*d*) Zondek, B.: Am. J. Obst. & Gynec. **33**: 979, 1937. Migliavacca.[81]

87. Gardner, W. U., and Allen, E.: Endocrinology **21**:727, 1937.

88. (*a*) Burrows, H., and Kennaway, N. M.: Am. J. Cancer **20**:48, 1934. (*b*) Burrows, H.: J. Path. & Bact. **41**:43, 1935. (*c*) Gardner and others.[72b]

89. Weinstein, L.; Gardner, W. U., and Allen, E.: Proc. Soc. Exper. Biol. & Med. **37**:391, 1937.

soon after the start of injections, the cells of the lumen and glands of the fundus became laden with glycogen [90] and persisted in such a state for long periods.[71b]

(*d*) Myometrial and stromal reactions. The development of uterine fibroids has been associated with clinical indications of excessive ovarian stimulation.[91] Stromal reactions have already been mentioned in connection with abnormal uterine reactions. Myometrial hyperplasia also occurred when estrogens were injected into young rats.[92] Benign fibromyomatous tumors frequently developed in the uteri of guinea pigs given large amounts of estrogens for several months.[73a] The tumors, single or multiple, were of various sizes and consisted of organized muscular and fibrous tissue lying below the peritoneal surface of the uterus.

Myometrial or stromal tumors have not been reported in the uteri of species other than the aforementioned one following the injection of estrogens. General hypertrophy and myxomatous transformation of the connective tissues of the cervix and upper part of the vagina occurred in mice receiving estrogens.[72b]

RESPONSES OF THE MALE TO ESTROGENIC SUBSTANCES

Estrogens, so-called because of their capacity to reproduce the changes occurring at estrus in normal female animals, stimulate certain reactions in males as well. They are essentially ambisexual in character.[93] The estrogens and androgens are certainly not sex specific in man, as both are usually excreted from the same persons.[94] It has been suggested that estrogens play some role in reproductive physiology in males.[95] The reactions of males to these substances in experiments of a chronic nature have been studied extensively. Malignant tumors developed in males only in the mammary glands, as previously described, and benign tumors only in the pituitaries and possibly testes of mice. However, other abnormal responses of a hyperplastic nature have been observed in males which have received estrogens.

Testes.—Since the experiments of Moore and Price [96] it has been generally considered that gonadotropic factors inhibit the activity of the

90. Overholser, M. D., and Nelson, W. O.: Proc. Soc. Exper. Biol. & Med. **34**:839, 1936.

91. Witherspoon, J. T.: Surg., Gynec. & Obst. **61**:743, 1935; Am. J. Obst. & Gynec. **31**:173, 1936.

92. Barks, O. L., and Overholser, M. D.: Anat. Rec. **70**:401, 1938.

93. Parkes, A. S.: Nature, London **141**:36, 1938.

94. Koch, F. C.: J. Urol. **35**:382, 1936. Dingemanse, E.; Brochardt, H., and Laqueur, E.: Biochem. J. **31**:500, 1937. Gallagher, T. F.; Peterson, D. H.; Dorfman, R. I., and Koch, F. C.: J. Clin. Investigation **16**:695, 1937.

95. Korenchevsky, V.: Brit. M. J. **2**:896, 1937.

96. Moore, C. R., and Price, D.: Am. J. Anat. **50**:13, 1932.

homologous or heterologous gonads by their action on the pituitary: a reciprocal pituitary-gonad relationship. In the male this concept applies to both the spermatogenic and the endocrine functions of the testis.

The cutaneous application of equilin and dihydroxyestrin (estradiol) to mice caused hypertrophy of the interstitial cells of the testes.[97] This change did not occur in mice receiving other estrogens with the exception of trihydroxyestrin. Later it was found that the change occurred in the testes of mice receiving other estrogens but more frequently in those receiving the more active chemicals or the higher concentrations. Equilin and estradiol in 0.1 per cent solution in benzene produced hypertrophy of interstitial cells in 69.5 and 86.7 per cent of the mice, respectively, whereas estrone in 0.01 per cent solution resulted in this change in only 30.7 per cent of the mice. The duration of treatment before the appearance of testicular change was shortest in mice receiving estradiol. An equal proliferation of the interstitial cells occurred in both testes. The changes were progressive, the cells first enlarging and separating the atrophic tubules. Later the interstitial cells occupied the greater part of the testis, altering its normal shape and giving it a yellow color.

Similar changes occurred in the testes of mice of one strain following the injection of hydroxyestrin benzoate and ketoestrin benzoate or equilin benzoate (500 international units and 0.1 mg. weekly, respectively).[98] Mice from four other strains similarly treated did not respond in the same manner. Smaller amounts or less active chemicals were ineffective. The seminiferous tubules decreased in size, and the testes became progressively smaller in the mice of the latter strains. Though the tubular damage was equally great in the former strain, the gross size of the testes was not appreciably altered, being compensated for by the hypertrophy of the Leydig cells. The tubules were completely replaced in the greater part of the testes in 3 mice receiving equilin benzoate. Mitotic figures were extremely rare, but it was assumed that these changes must have resulted from considerable hyperplasia.

The mice bearing the testes containing large amounts of glandular interstitial cells usually exhibited no evidence of masculinizing influences. One case was noted in which a male mouse acquired resistance to estrogens.[99] The sexual changes of the male accessory genital tissues occurring in mice receiving estrogens were altered, but in the case of the mouse mentioned a return to the normal condition occurred during the course of treatment. An adenomatous interstitial cell mass was found in one testis.

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97. Burrows, H.: J. Path. & Bact. **41**:218, 1935. Burrows.[33]
98. Gardner, W. U.: Anat. Rec. **68**:339, 1937.
99. Burrows, H.: J. Path. & Bact. **44**:699, 1937.

Seminal Vesicles and Prostate.—Because of the inhibitory effect of "sex hormones" on the gonads, these chemicals were once considered to be inhibitory to the heterologous accessory genital tissues. This is not entirely true, however, as these hormones, under experimental conditions at least, are ambisexual. The smooth muscles and connective tissues of the seminal vesicles of male rats not operated on and castrate male rats showed hypertrophy as a result of injection of estrogens.[100] The seminal vesicles of castrated or immature rats so treated increased in size. Estrogens administered simultaneously with androgens augmented the weight of the seminal vesicles beyond that obtained with androgens alone.[101] The prostatic changes were similar but usually less extensive. The epithelial tissues were not altered in castrate animals.

Prolonged injections of estrogens had no stimulating effect on the epithelium of the prostate or seminal vesicles in rats.[102] On the other hand, the epithelium of the seminal vesicles of mice underwent squamous metaplasia,[103] beginning at the proximal end and continuing distally in an irregular manner throughout the body of the gland. Metaplastic changes occurred earlier and more frequently in the prostate, being particularly pronounced in the coagulating gland.[104] The coagulating glands occasionally became tremendously enlarged by distention with accumulated cornified cells and invading leukocytes.[105] The small periurethral or prostatic glands in mice sometimes underwent metaplasia and occluded the urethra. The changes were not specific for any particular estrogenic chemical. The responses were similar in animals which received the estrogens cutaneously or subcutaneously. Comparatively large amounts, however, were required in intact animals.

The seminal vesicles and prostates of sexually inactive ground squirrels increased in size following the injection of estrogens.[106] The changes were largely due to fibromuscular hypertrophy, though a metaplastic stratification occurred in the prostatic glands.

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100. (*a*) Freud, J.: Biochem. J. **27**:1438, 1933. (*b*) David, K.; Freud, J., and de Jongh, S. E.: ibid. **28**:1360, 1934. (*c*) Korenchevsky, V., and Dennison, M.: ibid. **28**:1474, 1934; J. Path. & Bact. **41**:323, 1935. (*d*) Overholser, M. D., and Nelson, W. O.: Anat. Rec. **62**:247, 1935.

101. Korenchevsky, V., and Dennison, M.: Biochem. J. **28**:1486, 1934.

102. (*a*) Weller, D.; Overholser, M. D., and Nelson, W. O.: Anat. Rec. **65**: 149, 1936. (*b*) Moore and Price.[96] (*c*) Korenchevsky and Dennison.[100c]

103. (*a*) Burrows, H.: Am. J. Cancer **23**:490, 1935. (*b*) Weller and others.[102a]

104. Lacassagne, A.: Compt. rend. Soc. de biol. **113**:590, 1933. Lacassagne, A., and Villela, E.: ibid. **114**:870, 1933. Burrows, H.: J. Path. & Bact. **41**: 423, 1935. de Jongh, S. E.: Acta brev. Neerland. **5**:28, 1935. Burrows and Kennaway.[88a] Burrows.[103a] Weller and others.[102a]

105. Burrows and Kennaway.[88a] Burrows.[103a]

106. Wells, L. J.: Anat. Rec. **64**:475, 1936.

The prostate of dogs were enlarged up to eight times the size of controls.[107] Daily injections of 800 to 2,000 international units of estrogen were given for from 5½ to 7 weeks to 4 young male dogs, 2 castrated and 2 not operated on. The enlargement was the result of extensive prostatic epithelial metaplasia with cornification and fibromuscular hypertrophy. The microscopic pathologic description of these experiments was presented largely by Zuckerman and Groome [108] in a discussion of the etiology of benign prostatic hypertrophy. The extent of epithelial changes was inversely related to the extent of fibromuscular hypertrophy. The prostatic utricle and prostatic urethra also shared in the metaplastic stratification. Similar changes were found in the prostates of dogs bearing "feminizing" testicular tumors.[109]

The simultaneous administration of androgenic hormones, androsterone (3-cis-hydroxy-17-keto-androstane), dehydroandrosterone (3-cis-17-dihydroxy-androstane) and testosterone, partially or completely prevented the changes induced by the estrogens when these were given alone.[110] Testosterone was most effective. The fibromuscular changes were more difficult to control than the epithelial metaplasia. One milligram of progesterone given twice weekly inhibited the metaplasia of the prostate in the noncastrated males given estrogens but was not effective in castrated males.[111] Rusch [112] found that 1,250 micrograms of progesterone administered twice weekly to mice was ineffective but that 250 micrograms of testosterone prevented the epithelial changes when given simultaneously with estrogen.

The clinical interest in prostatic hypertrophy and its associated urologic complications led to a study of the influence of estrogens on the male monkey's genital tract. After immature male monkeys had been given subcutaneously a total of from 3,000 to 6,000 rat units of theelin during periods up to 42 days, the seminal vesicles weighed up to five and a half times as much as the controls.[113] The fibromuscular tissue of the cranial and to a lesser extent the caudal lobe of the prostate increased and the epithelium of the prostatic utricle, colliculus seminalis and cavernous urethra became thickly stratified. Similar observations were simultaneously reported by Parkes and Zuckerman [114] and Courrier and

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107. de Jongh, S. E., and Koh, D. S.: Acta brev. Neerland. **5**:177, 1935; **6**:46, 1936.

108. Zuckerman, S., and Groome, J. R.: J. Path. & Bact. **44**:113, 1937.

109. Greulich, W. W., and Burford, T. H.: Am. J. Cancer **28**:496, 1936. Zuckerman, S., and McKeown, T.: J. Path. & Bact. **46**:7, 1938.

110. Waterman, L.: Acta brev. Neerland. **6**:56, 1936. van der Woerd and de Jongh, S. E.: Acta brev. Neerland. **6**:88, 1936.

111. Burrows, H.: Nature, London **138**:164, 1936.

112. Rusch, H. P.: Endocrinology **21**:511, 1937.

113. Van Wagenen, G.: Science **81**:366, 1935; Anat. Rec. **63**:387, 1935.

114. Parkes, A. S., and Zuckerman, S.: Lancet **1**:925, 1935.

Gros.[115] The prostatic utricles of five species of subhuman primates are lined by a low stratified epithelium rather than a glandular, uterine-like mucosa as in man.[116] The response of these animals to estrogen was found to be epithelial stratification and cornification and fibromuscular hypertrophy. The prostatic utricle of Entellus langur, however, underwent glandular hyperplasia. The hypertrophy of the prostate was later assumed to be largely attributable to an increase of the utricular constituents proper. After injection of estrogens for a year the prostatic changes were not essentially different from those previously described.[117]

As in rodents, the prostatic changes induced by estrogens could be prevented by injecting androgens simultaneously.[118] The immature monkeys given daily injections of 200 micrograms of estrone and 2.5 mg. of androsterone showed prostatic changes of the same type as those receiving similar amounts of estrone alone, while 3 animals which received 100 international units of estrone and 5 mg. of androstendiol (3-trans-17-dihydroxy-5-androstene) daily showed no effects of estrogens but changes that might be attributable to the androgen alone. Progesterone did not alter the effect of estrogens. Testosterone propionate was more effective than the other androgens in preventing the prostatic changes occurring in monkeys given estrogens.[118]

Estrogens appear to have a marked stimulating effect on the fibromuscular tissues of the seminal vesicles and at least the utricular portion of the prostate but stimulate epithelial cell proliferation only in the prostatic utricle and certain parts of the urethra. The absence of epithelial hypertrophy in the prostate in monkeys studied contrasts with the responses to estrogens observed in dogs and mice.

The prostatic utricle apparently rarely develops in mice, as very few cases of hypertrophy have been observed in this species.[108a] The prostatic utricles of immature and of castrated or noncastrated adult guinea pigs underwent squamous stratification following injections of estrogen.[119] The changes were greatest in immature and castrated animals, indicating that the presence of the testes inhibited the action of estrogen.

Progressive metaplasia of the ducts and bodies of the bulbourethral glands of mice occurred when estrogens were applied cutane-

115. Courrier, R., and Gros, G.: Compt. rend. Soc. de biol. **118**:686, 1935.

116. Zuckerman, S., and Parkes, A. S.: (*a*) J. Anat. **69**:484, 1935; (*b*) Lancet **1**:242, 1936.

117. Zuckerman, S.: J. Anat. **72**:264, 1938. Zuckerman, S.: Lancet **1**:135, 1936.

118. Zuckerman, S.: Lancet **1**:1259, 1936. Zuckerman and Parkes.[116b]

119. Courrier, R., and Cohen-Solal, G.: Compt. rend. Soc. de biol. **121**:903, 1936.

ously in benzene.[120] Leukocytic invasion and occasionally abscess formation were associated with epithelial changes. Abnormal cysts or vesicles lined with keratinized epithelium were found in 17 of 342 mice following administration of estrogens.[121] It was assumed that these structures were of müllerian origin. They were located dorsal to the prostatic urethra, near the ends of the vasa deferentia or near the epididymis. The epithelial and stromal elements of the hydatids of Morgagni hypertrophied in several species of monkeys given estrogens.[122]

OTHER EFFECTS OF ESTROGENS

Pituitary Adenomatosis in Animals Receiving Estrogens.—The pituitary of the female rodent is usually larger than that of the male. The pituitaries of male or female rats may be increased in size by administering estrogens.[123] The extent of the hypertrophy depends in part on the amount and duration of treatment.

Almost simultaneously three investigators reported adenomatous changes in the pituitaries of rats or mice receiving estrogens. Hemorrhagic chromophobe adenomas developed in 3 of 12 mice reveiving cutaneous applications of estrone in benzene for periods up to 44 weeks. Simple enlargement of the pituitaries was observed in 8 other mice.[24a] Similar adenomatous changes of the pituitary have been observed by Zondek [124] and by McEwen, Selye and Collip.[56] On the other hand, Burrows [125] found only a single hypophysial tumor among 679 mice treated with various estrogenic chemicals. No pituitary adenoma was observed in over 100 mice receiving hydroxyestrin benzoate or ketoestrin benzoate,[28] though chromophobe adenoma developed in a mouse which had bilateral granulosa cell carcinoma.[126]

Lacassagne [31b] observed changes similar to those just cited in the pituitaries of mice receiving estrone, namely, increased vascularity and size of the anterior lobe, hypersecretion of colloid and general degranulation, particularly of the acidophilic cells. His previous failure to observe these changes was ascribed to the high concentration of equilin

120. Burrows, H.: J. Path. & Bact. **44**:481, 1937.

121. Burrows, H.: Proc. Roy. Soc., London, s. B **118**:485, 1935.

122. Zuckerman, S., and Krohn, P. L.: Phil. Tr. Roy. Soc. London, s. B **228**: 147, 1937.

123. (*a*) Hohlweg, W.: Klin. Wchnschr. **13**:92, 1934. (*b*) Selye, H.; Collip, J. B., and Thomson, D. L.: Proc. Soc. Exper. Biol. & Med. **32**:1377, 1935. (*c*) Wolfe, J. M.: ibid. **32**:757 and 1192, 1935; (*d*) Wolfe, J. M., and Chadwick, C. S.: ibid. **34**:56, 1936.

124. Zondek, B.: Lancet **1**:776, 1936.

125. Burrows, H.: Am. J. Cancer **28**:741, 1936.

126. Gardner, W. U.; Smith, G. M., and Strong, L. C.: Am. J. Cancer **26**: 541, 1936.

in his earlier preparations. Equilin alone produced the same changes, but they were greatly delayed. Equilenin was quite inactive.

Pituitary tumors developed in 29 of 35 male and female rats which were given biweekly injections of 5,000 mouse units of estradiol benzoate for periods of 8 months or more.[127] Some of these tumors weighed as much as 300 mg.; 19 of them averaged 149.3 mg. The duration of the period of injections was important. The pituitaries of male rats treated for 4 months were enlarged to about three times (26.08 mg.) their normal size, while the pituitaries of females were only slightly enlarged (11.7 mg.). At 7 to 9 months the response was similar in both males and females. With smaller amounts of estradiol benzoate (500 mouse units administered cutaneously in benzene twice weekly) similar changes occurred after a slightly longer period of treatment. The duration of treatment rather than the amount of active substance appeared of importance. Unlike other tumors observed, the tumors were considered to be of chromophilic cells. Adenoma of the pituitary has been recently observed in old female rats, along with other abnormal cellular changes.[128]

Lymphoid and Connective Tissue Tumors in Mice Receiving Estrogens.—As previously stated, hydrocarbons were determined to be carcinogenic largely by the response of tissues to which they were applied directly. Papillomas or skin tumors did not develop in mice which received estrogens cutaneously.[129] Spindle cell sarcoma developed, however, at or near sites of injection of estrogens in subcutaneous tissues.[130] Cori [6] observed spindle cell carcinoma in a mouse which had received a crude estrogenic substance in oil. Two sarcomas and 9 carcinomas developed in 16 castrated male mice bearing ovarian grafts.[8]

Five mice from two litters which had received theelin in aqueous solution and later ketoestrin benzoate in solution in oil showed spindle cell sarcoma.[130a] The lesions arose at the sites of injection of the oily solution of estrogen. Later 2 other tumors of this type developed in animals receiving hydroxyestrin benzoate.[28] These tumors were observed among 111 mice which had received various estrogens in larger amounts for 125 days or more. Lacassagne observed 5 spindle cell sarcomas in mice which had been treated with (*a*) equilin, (*b*)

127. Zondek, B.: Am. J. Cancer **33**:555, 1938.

128. Wolfe, J. M.; Bryan, W. R., and Wright, A. W.: Proc. Soc. Exper. Biol. & Med. **38**:80, 1938.

129. Burrows.[33] Burrows.[88b] Burrows.[103a]

130. (*a*) Gardner, W. U.; Smith, G. M.; Strong, L. C., and Allen, E.: Arch. Path. **21**:504, 1936. (*b*) Lacassagne, A.: Compt. rend. Soc. de biol. **126**:190, 1937. (*c*) Burns, E. L.; Suntzeff, V., and Loeb, L.: Am. J. Cancer **32**:534, 1938. Gardner.[28]

estrone, equilin and equilenin and (*c*) equilin and sheep pituitary extract. Four of these tumors occurred in the subcutaneous tissues at sites of injection. One occurred in the bladder.

More recently 10 sarcomas developed in 247 mice which had received estrone, estrone and progestin, progestin and liver extract for 12 to 18 months.[130c] Sarcoma was not observed in 128 control mice. The fact that tumors developed in 2 mice receiving progestin and liver extract, respectively, which did not receive estrogens, indicates that the response was not specific for the latter chemicals. All the growths appeared at or near sites of injection with the exception of a tumor which developed in the vaginal wall.

TABLE 7.—*Development of Spindle Cell Sarcoma in Mice Receiving Estrogens*

Mice Treated	Mice Showing Tumors	Age at Development of Tumors, Days	Substance Used in Treatment	Location	Investigator
?	5	188-273	Estrone Ketoestrin benzoate	Sites of injection	Gardner, Smith, Strong and Allen [130a]
111	7 (includes 5 above)	188-273	Estrone Ketoestrin benzoate Hydroxyestrin benzoate	Sites of injection	Gardner [28]
?	5	358-523	Equilin Estrone, equilin, equilenin Equilin and pituitary extract	Sites of injection (bladder)	Lacassagne [130b]
247	10	12-18 m.	Estrone Estrone and progestin Progestin Liver extract (in 1)	Sites of injection 1 rhabdomyoma	Burns, Suntzeff and Loeb [130c]
128	0	.....	Control, untreated	.....	Burns, Suntzeff and Loeb [130c]
77	1	208	Untreated Oil of benné (in 1) Cholesterol and cholesterol benzoate	.....	Gardner (unpublished data)

None of 236 mice which had received subcutaneous injections of various fats, fatty acids or oils showed tumors at the sites of injection.[131] One mouse which had received weekly injections of 0.05 cc. of sesame oil presented spindle cell sarcoma at the site of injection, while sarcoma did not appear in 76 other mice which had received sesame oil alone or containing cholesterol or cholesterol benzoate.[37]

These experiments indicate that estrogens may have some effect on the development of sarcoma. The incidence of tumors of this type is low, however, and a few tumors (3 of the 23 described in table 7) developed in mice which had received nonestrogenic materials.

Lymphoid tumors, particularly leukemias, occur frequently in certain strains of mice. In other strains they occur rarely. In 111 mice

131. Burrows, H.; Heiger, I., and Kennaway, E. L.: J. Path. & Bact. **43**: 419, 1936.

which had received estrogens 3 lymphoid tumors (1 leukemic and 2 sarcomatous) were found.[28] The leukemia was transplanted and maintained for many generations.[132] Each of the 2 lymphosarcomas arose in the mediastinum and invaded the lungs and intercostal region. One of these tumors was grafted into other mice of the same strain and has continued to grow as a localized mass, preserving its original histologic character. The two original lymphoid tumors had developed in mice of two different strains.

Lymphoid tumors developed in 14 mice from four different strains on administration of estrogens;[133] 11 of the mice exhibited extensive involvement of the mediastinum. Of the tumors, 2 arose in a strain which is characterized by a high incidence of mammary tumor and 12 in strains characterized by a low incidence. Estrone was used, alone and in combination with equilin, equilenin, progesterone or anterior pituitary extracts. Tumors of this type had not developed in any untreated mice in the colony.

Since the initial observations mentioned,[28] lymphoid tumors have developed in 19 other mice which have received large amounts of estrogenic substance.[134] Four of these mice, all of the C_3H strain, received equilin benzoate (the data are included in table 6). In this strain lymphoid tumors have not developed in any of 77 mice which have been observed throughout life and have received no treatment or injections of sesame oil or solutions of cholesterol or cholesterol benzoate in sesame oil.

The increased incidence of lymphoid tumors in mice receiving estrogens indicates that estrogens may be in some way related to the development of such neoplastic changes.

Other Effects of Large Amounts of Estrogen on the Body.—Estrogens when administered in large amounts over long periods are decidedly toxic. The changes accompanying such treatment in the pituitary, accessory genital tissues and gonads have been previously mentioned. Estrogens will also inhibit body growth.[135] Many mice die from uterine or prostatic inflammatory processes or as a result of urinary complications following retention of urine.[26] The thymus rapidly involutes, and the adrenal hypertrophies, shortly after an initial injection of estrogen.[136]

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132. Lawrence, J. H., and Gardner, W. U.: Am. J. Cancer **33**:112, 1938.

133. Lacassagne, A.: Compt. rend. Soc. de biol. **126**:193, 1938.

134. Gardner, W. U.; Smith, G. M.; Strong, L. C., and Allen, E.: To be published.

135. Zondek, B.: Lancet **2**:842, 1936; Folia clin. orient. **1**:1, 1937.

136. Schacher, J.; Browne, J. S. L., and Selye, H.: Proc. Soc. Exper. Biol. & Med. **36**:488, 1937.

Recently profound skeletal changes have been observed in pigeons, mice, rats and chickens receiving estrogens.[137] These changes have been most extensively studied in mice. The pubis may be largely resorbed and the symphysis replaced by a ligament.[137a] At the same time new endosteal bone forms throughout the rest of the skeleton, which under certain conditions largely replaces the marrow.[137b] The tendency for the marrow tissue to be replaced results in profound anemia in these mice. Pybus and Miller[137d] recently described an earlier development of osteogenic sarcoma in a strain of mice given estrogens. The changes of the reticular tissues of the body have not been studied in detail in animals receiving estrogens.

The adrenals have been studied extensively by several investigators. A degenerative change occurred in adrenals of about one half of the mice receiving large amounts of estrogen. Masses of lipoid-containing cytoplasmic material and scattered pyknotic nuclei occupied variable areas of the deeper parts of the cortex.[138] Similiar degenerative areas in the adrenal cortex were observed by Cramer in animals which had received estrogens. Later, it was reported that these changes occurred spontaneously in old females of a strain of mice highly susceptible to mammary tumors.[139] As these changes were not observed in stock mice up to 9 months of age in which mammary tumors rarely developed, they were associated with the tendency toward development of mammary tumors. Also, on this basis, inbred mice were assumed to be abnormal animals.

Estrogens apparently alter directly or indirectly certain extragenital functions of the body. In most instances the details of the mechanism of many of these changes are not completely understood. The functional interrelationship of the various endocrines adds to the complexity of the problem.

INFLUENCE OF THE GONADS AND "SEX HORMONES" ON THE GROWTH OF TUMORS

Influence of Ovaries on Growth of Tumors.—Though ovarian endocrine function must have been associated with the growth of certain benign mammary tumors at an early date, little work was done until comparatively recent times on the influence of the ovary on tumor

137. (*a*) Gardner, W. U.: Am. J. Anat. **59**:459, 1936. (*b*) Gardner, W. U., and Pfeiffer, C. A.: Proc. Soc. Exper. Biol. & Med. **37**:678, 1938; **38**:599, 1938. (*c*) Pfeiffer, C. A., and Gardner, W. U.: Endocrinology **23**:485, 1938. (*d*) Pybus, F. C., and Miller, E. W.: Nature, London **142**:872, 1938.

138. (*a*) Burrows, H.: J. Path. & Bact. **43**:121, 1936. (*b*) Lacassagne, A., and Raynaud, A.: Compt. rend. Soc. de biol. **124**:1183, 1937; (*c*) ibid. **124**: 1186, 1937.

139. Cramer, W., and Horning, E. S.: J. Path. & Bact. **44**:633, 1937.

growth. In 1901 Loeb observed that a mammary adenoma on a rat grew rapidly during pregnancy and that it failed to recede after parturition as did the normal mammary tissue. Pregnancy, however, did not alter appreciably the rate of growth of mammary carcinoma in mice.[5e]

The rate of growth, type of growth and incidence of successful "takes" of a transplanted mammary fibroadenoma varied greatly, depending on the sex and on the stage of reproductive life of the rats.[140] In young and in aged rats the transplanted tumors were predominantly fibrous, while in sexually active females the glandular tissues predominated. The incidence of successful grafts was 66 per cent in females and 33 per cent in males, and following castration it was 16 per cent and 66 per cent, respectively. Castration in the male increased the rate of growth as well as the incidence. During pregnancy the transplants grew rapidly and frequently became pure adenoma. Some of the tumors secreted. Similar observations have been made by other investigators,[141] and Emge[142] reviewed the subject from the standpoint of both clinical and animal material. Pregnancy did not uniformly affect the rate of growth of certain types of tumors, adenofibroma, fibroma and sarcoma. A table was given showing the observations of several investigators. Rapidly repeated pregnancies did not uniformly increase the rate of growth of a mammary adenofibroma (5-B1) nor did repeated pregnancies alter the morphologic character to a greater extent than a single pregnancy.[143] Pregnancy did not alter the rate of growth of transplants of three different types of sarcoma.[144]

Transplants of an ovarian carcinoma, which produced estrogenic material, invariably grew in female mice but grew infrequently in males and then only at a reduced rate.[145] The sex of the host may affect the development of certain tumors, particularly certain benign mammary tumors, but the response is not necessarily uniform.

Influence of Ovarian and Gonadotropic Substances on Tumor Growth.—Gonadotropic hormones have slight or no effect on the rate of growth of tumors, as indicated by several investigations that have been undertaken. Slight increases in the numbers of successful transplantations of mammary fibroadenoma were observed following injection of the gonadotropic substance from the urine of pregnant women

140. (*a*) Heiman, J.: Am. J. Cancer **22**:497, 1934. (*b*) Heiman, J., and Krebhiel, O. F.: ibid. **27**:450, 1936.

141. Grauer, R. C., and Robinson, G. H.: Am. J. Cancer **16**:191, 1932.

142. Emge, L. A.: Am. J. Obst. & Gynec. **28**:682, 1934.

143. Emge, L. A., and Murphy, K. M.: Proc. Soc. Exper. Biol. & Med. **37**: 620, 1938.

144. Emge, L. A.; Schilling, W., and Wulff, L. M. R.: Proc. Soc. Exper. Biol. & Med. **38**:388, 1938.

145. Strong, L. C.; Pfeiffer, C. A.; Hill, R. T., and Gardner, W. U.: Genetics **23**:585, 1938.

(antuitrin S) and theelin in combination to castrate male or female rats, though the morphologic aspects of the tumor were not changed.[140b] The injection of gonadotropic substances or of estrogen did not appreciably alter the growth of sarcoma types R 10 and 180.[146] The administration of sufficient estrogen to cause dwarfism did not retard the rate of growth of transplants of tumors induced by benzpyrine.[147] The daily injection of 0.4 rabbit units of a corpus luteum factor accelerated the rate of growth of transplants of Flexner-Jobling carcinoma and Fujinawa sarcoma.[148] The effect was more striking in ovariectomized animals. In general, the addition of ovarian and gonadotropic substances has little effect on the growth of malignant tumors, the observations paralleling those obtained in normal and ovariectomized hosts.

Influence of Sex on the Response to Carcinogenic Chemicals.—The action of carcinogenic tars or chemicals has been determined largely by the effects following cutaneous application of these substances to laboratory rodents. Several rodent variables, such as age, strain, sex and susceptibility to mammary cancer, have been studied in connection with the response to carcinogens. Of these, the effects of sex and of the susceptibility to development of mammary tumors will be considered.

The earlier investigators found that sex had little or no effect on the incidence of tumors following applications of tar.[149] Cutaneous applications of methyl cholanthrene dissolved in benzene to normal and castrate male and female mice revealed no influence of sex on the incidence of tumors.[150] On the other hand, Bonser [151] found that warts following cutaneous applications of tar or methyl cholanthrene developed sooner and more frequently became malignant in females. The sex influence on the tar cancer varied in different strains. The tar warts of males of all strains persisted longer before malignant growths developed. The tendency for mammary tumors to develop could not be associated uniformly with response to carcinogenic agents. Kreyberg [152] observed that male mice showed a distinctly later appearance of tar tumors than females. Twelve hundred mice from strains varying in

146. Bischoff, F., and Maxwell, L. C.: Am. J. Cancer **27**:87, 1936.

147. Zondek, B.: Lancet **1**:689, 1937.

148. Nitta, Y.: Jap. J. Obst. & Gynec. **19**:512, 1936.

149. (*a*) Fibiger, J.: Deutsche med. Wchnschr. **47**:1449, 1921. (*b*) Roussy, G.; Leroux, R., and Peyre, E.: Presse méd. **30**:1061, 1922.

150. Gilmour, M. D.: J. Path. & Bact. **45**:179, 1937.

151. Bonser, N. M.: J. Path. & Bact. **46**:581, 1938.

152. Kreyberg, L.: Am. J. Cancer **24**:554, 1935; in A Symposium on Cancer: Addresses Given at an Institute on Cancer Conducted by the Medical School of the University of Wisconsin, Madison, Wis., University of Wisconsin Press, 1938, pp. 3 and 183.

susceptibility to the development of mammary tumors were used. One strain in which breast cancer had not developed showed earlier appearance and higher incidence of tar cancer than another strain in which mammary tumors developed frequently.

Though the incidence of tar tumors was similar in males and females, the period of treatment was longer in the former.[153] Mice from a strain with a high incidence of mammary tumor acquired tar cancers much sooner than those from a line having a low incidence of tumor, though the incidence was similar.

The simultaneous application of a carcinogenic agent and estrogens has also given somewhat variable results. In 1 of 16 female mice a mammary cancer and in 11 tar tumors developed following weekly injections of an estrogen in oil and tar painting.[154] This investigator concluded that estrogens had little effect on the action of tar. On the other hand, Gilmour [150] found that the incidence of tumors was slightly higher in mice which were painted with estrogens dissolved in chloroform and with methyl cholanthrene than in mice which received the carcinogen alone.

Using a nontumor-bearing stock, Perry and Ginzton [27] observed 4 mammary tumors among 37 immaturely ovariectomized mice which were painted with 1,2,5,6-dibenzanthracene dissolved in oil, while 7 mammary tumors developed in a similar group of mice receiving estrogens simultaneously.

The influence of estrogens on the action of carcinogens has been indicated. Estrogens may increase the activity of some of these chemicals, but the changes are apparently slight. The susceptibility of mice of different strains to carcinogenic chemicals does not seem to be more than incidentally associated with the tendency to have mammary tumors.

ARE ESTROGENS CARCINOGENIC?

Few investigators have openly committed themselves on this point. In fact, the term "carcinogenic" is not necessarily too clearly defined. These points have recently been discussed by Dodds [155] and Cramer.[156] One might quote statements from these two papers:

> There is no experimental connection between the observations with compounds like 1:2:5:6 dibenzanthracene and the production of tumors in the breast by oestrin. At present we are completely ignorant as to the mechanism whereby oestrin produces cancer of the breast in the mouse. It seems unlikely that it is by the same process that a tumor is produced in the skin of a mouse by painting with 1:2:5:6 dibenzanthracene.[155]

153. Reinhard, M. C., and Candee, C. F.: Am. J. Cancer **16**:640, 1932.
154. Kreyberg, L.: Nord. med. tidskr. **12**:1236, 1936.
155. Dodds, E. C.: Acta, Union internat. contre cancer **1**:332, 1936.
156. Cramer, W.: Am. J. Cancer **30**:318, 1937.

Cramer [156] comments as follows:

> We are equally ignorant as to the mechanism whereby the carcinogenic hydrocarbons produce cancer of the skin. In the mamma, as in the skin, the development of cancer is preceded by a hyperplasia, and in both organs the development of cancer sets in after a variable but equally prolonged period of time has elapsed.

Another group of investigators included estrogens in their discussions on chemical compounds as carcinogenic agents.[2]

Hereditary factors are of great importance in determining the influence of estrogens on mammary cancer. They also play a significant role in determining responses to the generally recognized carcinogenic chemicals. In view of the ignorance pertaining to the fundamental biologic activities of all these compounds with respect to the onset of uncontrolled growth, it seems premature to draw definite conclusions on this point. From the practical standpoint it seems that one must accept the conclusion that, as shown by the data cited, without estrogenic stimulation there are no mammary tumors in male mice and few uterine tumors. In animals receiving *long-continued* injections of *comparatively high* amounts such tumors appear. Hereditary factors are also of significance in determining the response of the mammary glands.

This question of the "carcinogenic" effect of estrogens is of great significance because of the therapeutic applications of this chemical. This point has been well discussed by Cramer [156] in view of the present state of information from experimental animals. He states, ". . . therapy extending over several years, . . . does involve such a risk, especially in susceptible individuals, that is to say in women with a family history of mammary cancer."

Notes and News

University News, Promotions, Resignations, Appointments, Death, etc.—At the University of Oslo, Francis Harbitz, having reached the age limit, has retired from the professorship of general pathology, pathologic anatomy and legal medicine. This professorship has been divided into two, with the appointments of Leiv Kreyberg as professor of general pathology and pathologic anatomy and of Georg Waaler as professor of legal medicine.

Howard McCordock, professor of pathology at the Washington University School of Medicine, died on Nov. 13, 1938, at the age of 43.

A department of oncology, with Stanley Reiman at the head, has been established in Hahnemann Medical College and Hospital of Philadelphia.

L. S. Dudgeon, professor of pathology at the University of London, dean of St. Thomas's Hospital Medical School and director of pathology and bacteriology at the hospital, died on Oct. 22, 1938.

L. L. Robbins has been appointed instructor in pathology in the University of Vermont.

Herbert U. Williams, former dean and professor of pathology and bacteriology in the University of Buffalo School of Medicine, died on Dec. 8, 1938, at the age of 72 years.

Mycopathologia.—This is a new international journal, devoted to medical mycology. It is published under the joint editorship of R. Ciferri, University of Florence, and P. Redaelli, University of Padua, by W. Junk, at The Hague. Articles are published in the language of the author, English, German, French, Italian or Spanish. Articles in the last two languages usually are followed by abstracts in English.

Society News.—The fiftieth annual meeting of the Association of American Medical Colleges will be held in Cincinnati, Oct. 23, 24 and 25, 1939.

The first conference of the Massachusetts Medico-Legal Society was held at the Mallory Institute of Pathology, Boston, on Oct. 4 and 5, 1938. The society is considering plans to make the conference an annual event with a three or four day program instead of the two day program of this year's session.

Obituaries

HOWARD A. McCORDOCK, M.D.

1895-1938

Late on Sunday night, Nov. 13, 1938, Dr. Howard A. McCordock, professor of pathology in Washington University School of Medicine, died suddenly from heart disease, the result of a rheumatic infection acquired during childhood. His colleages and associates were greatly shocked and grieved to learn of this sad event. At the early age of 43, when he was just entering into his fullest activities, he was taken away, only a few days after he had returned from a scientific meeting in Chicago.

Dr. McCordock was born in Brooklyn in 1895. He took the degree of Bachelor of Science at the University of Buffalo and graduated from the medical school of the same university in 1923. He served as instructor in pathology in this medical school for one year. From 1924 to 1927 he worked and taught at the Johns Hopkins University Medical School under a National Research Fellowship. In 1927 he came to the department of pathology of the Washington University School of Medicine, first as assistant professor; a year later he was made associate professor, and on the retirement of Dr. Leo Loeb he became, on Jan. 1, 1937, professor of pathology and head of the department.

While in the Johns Hopkins University Medical School, in conjunction with Dr. A. P. Rich, he carried out extensive studies on the relations between allergy and immunity in tuberculosis and on the pathogenesis of tuberculous meningitis. In St. Louis he made contributions to the study of the effects of iodine and thyroid substance on the thyroid gland and published observations concerning the reproduction of certain symptoms of exophthalmic goiter in guinea pigs by means of injections of extracts of anterior pituitary from cattle. He described the occurrence of nuclear occlusion bodies in the lungs in whooping cough. During recent years he concentrated on investigations of the St. Louis type of encephalitis. He was associated with Dr. Charles Armstrong and other workers of the United States Public Health Service in the original study of this disease soon after its first appearance in 1933, and he described very carefully the structural changes which are produced by the virus. He also showed that extensive visceral lesions can be produced in mice by intraperitoneal and intracerebral inoculations of homologous salivary gland virus, and he was greatly interested in the study of the interstitial type of pneumonia which is caused by various viruses. He was in the midst of further experiments relating to the virus of encephalitis when

his untimely death cut short this work. While these were his main lines of research, he also published reports concerning the action of paratyphoid bacilli and some interesting autopsy observations.

HOWARD A. McCORDOCK
1895-1938

Dr. McCordock was an excellent teacher. The students appreciated his interest in them and were quick to respond to his influence and advice. He was keenly interested in the clinical application of pathology.

For his clinical pathologic conferences he made most careful preparation, and these conferences were always stimulating. As a man he was quiet, thoughtful and kind. His interests were many-sided; he was an expert in several kinds of photography, and in appreciation of his contributions in this field he was elected to membership in the Royal Society of Photographers of England; his paintings in oil reveal fine sensitiveness to the beauties of nature. Yet, his greatest happiness was found in his home.

LEO LOEB.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

Experimental Pathology and Pathologic Physiology

Reduction of Intracranial Pressure by Concentrated Solutions of Human Lyophile Serum. J. Hughes, S. Mudd and E. A. Strecker, Arch. Neurol. & Psychiat. **39**:1277, 1938.

To reduce cerebrospinal fluid pressure, the authors used concentrated human blood serum which had been dried in a high vacuum from the frozen state. Such a dried serum is readily soluble (lyophile serum) and is used either in distilled water or in a 50 per cent solution of sucrose. The serum solution is given intravenously and the cerebrospinal fluid pressure recorded. The relative effectiveness of the serum solution was contrasted with that of a 50 per cent solution of sucrose in control normal subjects. The human lyophile serum solution proved to be more effective than the solution of sucrose, as it maintained the reduction of the cerebrospinal fluid pressure for longer periods. In addition, the concentrated lyophile serum caused an increase in the blood pressure and in some cases gave reactions resembling anaphylactic shock.

G. B. Hassin.

Reduction of Cerebrospinal Fluid Pressure by Concentrated Lyophile Serum. D. Wright, D. Bond and J. Hughes, Arch. Neurol. & Psychiat. **39**:1288, 1938.

Wright and his associates studied the effect of intravenous injections of concentrated serum on the pressure of the cerebrospinal fluid in dogs. Cisternal puncture was used. The recording was continuous; in one animal it lasted for eight hours. The extent and duration of the fall of the pressure depended on the dose injected. Thus, in animals which received 4 cc. of the serum per kilogram of weight the pressure was lowered 48 mm., and after eight hours of continuous recording it was 16 mm. below its initial level. The authors emphasize the fact that the results obtained from injections of serum were superior to those from injections of sodium chloride and sucrose.

G. B. Hassin.

Experimental Hypertension. H. Goldblatt, J. Exper. Med. **67**:809, 1938.

Hypertension in the acute malignant phase has been produced in 17 dogs. The method used was the same as for the benign type, namely, constriction of the main renal arteries or, which is the equivalent, constriction of the aorta above the origin of both main arteries, but the constriction was especially severe. The malignant phase was characterized by hypertension, terminal renal insufficiency and development of petechiae and larger hemorrhages in many internal organs, especially in the alimentary tract. These were due to dissecting hemorrhage through, or rupture of, the walls of severely hyalinized or necrotic arterioles and were more severe and more widespread in animals that had had benign hypertension for a period before the onset of renal insufficiency. In animals that had had benign hypertension for a long period, thickening of the media also occurred in arterioles, with or without hyalinization of the intima. Elevation of the blood pressure (mechanical factor) and renal insufficiency (humoral factor) are at least two of the conditions necessary for necrosis of arterioles and hemorrhages. Necrotic arterioles and hemorrhages have not yet been observed in animals that have had very high blood pressure for years without renal insufficiency, or in animals with

azotemia, due to removal of both kidneys, but without hypertension. Hyalinized retinal arterioles have been observed in dogs with persistent hypertension and with moderate or no disturbance of renal function. That ischemia is not the cause of the necrosis of the arterioles is shown by the absence of such necrosis from the ischemic kidneys of the dogs and the widespread presence of it in other organs that were not ischemic. These experiments show that the necrotic arterioles and hemorrhages are secondary to, and not the primary cause of, the malignant phase of hypertension. FROM AUTHOR'S SUMMARY.

TRANSPLANTATION OF LIVER TISSUE INTO THE ANTERIOR CHAMBER OF THE EYE. J. BOECK and H. POPPER, Virchows Arch. f. path. Anat. **299**:219, 1937.

Although various tissues have been transplanted into the anterior chamber of the eye, liver tissue has not been. It was tried in the experiments reported, with the view that gross inspection of the transplant in the living animal might permit detection of changes which could be confirmed by microscopic examination. Rabbits were used; a small fragment of liver was removed at laparotomy and introduced into the anterior chamber of the same animal. The eyes were enucleated at intervals of from two days to eight months and examined microscopically. Inflammatory reaction was evident within two days and by the fifth day had led to the formation of granulation tissue between the transplant and the iris. This tissue was richly vascularized, which accounted for the fact that necrosis of the transplant did not occur. No proliferation of liver cells was observed, but new bile ducts were formed, which arose not from the original interlobular ducts but from the precapillary bile ducts. The liver parenchyma degenerated and disappeared, being replaced by new-formed connective tissue. In later stages macrophages with brown pigment were numerous. O. T. SCHULTZ.

INFLUENCE OF THE NERVOUS SYSTEM ON HYPERERGIC INFLAMMATION. H. KAISERLING, Virchows Arch. f. path. Anat. **299**:253, 1937.

In earlier work, previously abstracted, Kaiserling described an acute ulcerative inflammatory reaction in rabbits sensitized to horse or swine serum, which followed injection into the interstitial or perifollicular lymphatics of the vermiform appendix of the provocative dose of the antigen. Section of the splanchnic nerve and resection of the paravertebral ganglions were later found to intensify the inflammatory reaction and to hasten its course. This apparent influence of the sympathetic nervous system was further studied with the aim of determining whether it might evoke a hyperergic inflammatory response under conditions that do not lead to such a response in the intact animal. The injection of the antigen into the lumen of the appendix was without effect in the sensitized rabbit. If however, the splanchnic nerves were sectioned and the paravertebral ganglions resected, paralytic hyperemia of the appendix, due to vasodilatation, was evident, and injection of the antigen into the lumen of the appendix was followed by an acute inflammatory reaction. Prolonged stimulation of the sympathetic nerves, which caused visible blanching, due to vasoconstriction, had no effect. Another series of sensitized rabbits received pilocarpine subcutaneously. At the height of the reaction to the pilocarpine vagi were sectioned in half of the animals and in the other half stimulated electrically, the antigen being injected into the lumen of the appendix. In the latter group acute hemorrhagic purulent appendicitis developed. Entirely negative results were obtained in animals in which the vagi had been sectioned. O. T. SCHULTZ.

CULTURE OF ADULT HUMAN HEMOPOIETIC TISSUES. R. MEIER, E. POSERN and G. WEITZMANN, Virchows Arch. f. path. Anat. **299**:316, 1937.

The authors describe the successful growth in vitro of adult human bone marrow and lymphoid tissue. For a comparative study, subperitoneal connective

tissue was also cultured. Embryo extract was not necessary for the culture of the tissue used. The character of the tissue is described briefly, in preliminary fashion, the analysis of cellular details being reserved for later presentation. The marrow cultures contained large cells with short processes. To what extent such cells have the hemopoietic potencies of bone marrow is left for future consideration. In cultures of lymphoid tissue there was an interlacing radial and circumferential growth of fine connective tissue fibrils that appeared to be characteristic for lymphoid tissue. In the meshes were round cells of lymphocytic type.

O. T. SCHULTZ.

RELATION OF THE VEGETATIVE AND PERIPHERAL NERVOUS SYSTEM TO HYPERERGIC INFLAMATION. H. KAISERLING, Virchows Arch. f. path. Anat. **301**:111, 1938.

This is a continuation of work previously reported from Klinge's laboratory, undertaken for the purpose of determining in what way and to what degree the hyperergic inflammatory reaction may be influenced by the nervous system. Rabbits were sensitized by injections of horse or swine serum. In some experiments after denervation of a kidney the provocative injection of the antigen was made either into the renal artery or into the general circulation. In other experiments the injection was made into the muscles of a thigh during prolonged electrical stimulation of the sciatic nerve, and in still others into the muscles of the thigh of a limb denervated as completely as possible. In each instance the opposite kidney or limb served as a control. Denervation of the kidney did not alter the qualitative character of the inflammatory reaction but caused the latter to run a more rapid and severe course. Denervation led to vasoconstrictor paralysis and passive congestion, the latter appearing to be the main factor in the more severe reaction. Stimulation of the sciatic nerve was without effect. Intramuscular injection of the antigen during the first hours or days after denervation of the hindlimb led to a more severe and more rapidly progressing inflammatory reaction, again because of vasoconstrictor paralysis and passive hyperemia. When the provocative injection was made some three or four weeks after denervation of the limb, the inflammatory reaction was the same as in the control limb. The nerves of the local inflammatory field participated in the mesenchymal reaction, but there was no evidence to support the view that the nerve is the primary point of attack in the hyperergic process.

O. T. SCHULTZ.

Pathologic Anatomy

THE CALCIFIED NODULAR DEFORMITY OF THE AORTIC VALVE. B. J. CLAWSON, J. F. NOBLE and N. H. LUFKIN, Am. Heart J. **15**:58, 1938.

The calcified nodular deformity of the aortic valve with stenosis is frequently not diagnosed because of the common occurrence of sudden death and the lack of constancy of symptoms and signs and because the symptoms are often similar to those of other types of heart disease, such as coronary sclerosis. This lesion is common. It is the usual kind of lesion found in healed aortic valve deformities. The healed, thickened leaflets of the aortic valve, as well as those of the mitral valve, commonly undergo calcification. The available evidence in the authors' material relative to the etiologic background supports a rheumatic origin of the lesion rather than a degenerative or an atherosclerotic one.

FROM AUTHORS' SUMMARY.

AN INJECTION PLUS DISSECTION STUDY OF CORONARY ARTERY OCCLUSIONS AND ANASTOMOSES. M. J. SCHLESINGER, Am. Heart J. **15**:528, 1938.

The coronary arteries in normal human hearts and even in senile hearts are true Cohnheim end arteries, without anastomotic connections; such anastomoses

do not develop pari passu with increase in age. Anastomoses always develop readily whenever and wherever arteriosclerotic narrowing or occlusion causes obstruction of the circulation in the coronary artery; these anastomoses are localized to the regions where they are needed.

To ascertain accurately the site and effects of all occlusions and anastomoses of the coronary arteries in the heart, it was necessary to devise a method capable of visualizing completely and simultaneously the entire course of all arterial branches so that they could be studied in detail. This has been accomplished in a simple standardized procedure which utilizes (*a*) a newly devised multicolored radiopaque injection mass, (*b*) a new method of cutting open the heart that has received the injection and (*c*) a complete dissection of the colored arterial tree. The new injection mass consists of a suspension of lead phosphate in agar, colored differently for the right and the left coronary artery. It is injected at a pressure of 150 mm. of mercury, at 45 C.; it sets quickly and permits immediate cutting and roentgenographic reproduction of the fresh, unfixed heart.

The new method of opening the heart unrolls all the coronary vessels so that they lie in one plane and avoids overlapping of the roentgenographic shadows of the injection mass within them. The distribution of the multicolored mass in the dissected branches of the coronary arteries gives an absolute index of the distribution of the blood from the orifice of either coronary artery.

From Author's Summary.

Cellular Reactions to Thorium Dioxide in Hepatosplenography. Wallace M. Yater and Eugene R. Whitmore, Am. J. M. Sc. **195**:198, 1938.

Sitxy-four necropsies have been made at intervals of a few days to three years after injection of an average dose of 75 cc. of thorium dioxide for the purpose of making a hepatosplenogram. In no case was there evidence of injury to the tissues or of a cellular reaction that could be ascribed to the presence of the thorium dioxide. A subcutaneous nodule was excised from the arm four years and five months after thorium dioxide had been injected accidentally into the subcutaneous tissues. The thorium dioxide was walled off by dense hyaline connective tissue, the nodule resembling those found in the lungs in nodular silicosis. Nowhere was there evidence of injury to the tissues or of cellular reaction other than the pimary reaction resulting in the walling-off of the thorium dioxide.

From Authors' Summary.

Developmental Defects at the Foramen Ovale. B. M. Patten, Am. J. Path. **14**:135, 1938.

Emphasis is placed on the necessity of discriminating more critically between an open foramen ovale with a competent but unfused valve, on one hand, and a frankly unguarded foramen ovale, with an incompetent valve, on the other. With an unfused but competent valve, transseptal leakage, if it occurs, is limited to one direction—from right to left. The common cause of such leakage is some disturbance of the pulmonary circuit which results in relative lowering of left atrial intake and pressure. What occurs at the foramen ovale in such cases should be regarded as a result of disturbances elsewhere and not as a cause. In sharp contrast with such cases are those in which the foramen ovale is inadequately guarded by an incompetent valve. In these cases, provided the pulmonary circuit is normal, transseptal flow appears to take place consistently from left to right. This overloads the right side of the heart at the expense of the left and causes characteristic changes from the normal cardiac proportions. There tend to be marked dilatation and hypertrophy, resulting in a great increase in the weight of the heart. The right side of the heart, especially the right ventricle, is most conspicuously involved, whereas the left ventricle remains strikingly uninvolved.

Consonant with the relative ventricular development, the pulmonary artery is markedly larger than the aorta, which tends to be below normal in size and thin walled. In these cases of an unguarded foramen ovale the characteristic and clinically recognizable changes in cardiac structure are clearly the result of the defect. Even in these cases the prognosis should not be unduly pessimistic, as many persons support such defects surprisingly well and live to an advanced age.

FROM AUTHOR'S SUMMARY.

SPONTANEOUS CARDIOVASCULAR DISEASE IN THE RAT. S. L. WILENS and E. E. SPROUL, Am. J. Path. **14**:177 and 201, 1938.

The manifestations of cardiac disease in a group of 487 inbred albino rats maintained on adequate diets and under constant laboratory conditions, and free from experimentation over their entire span of life, are described. Of the fourteen diseases classified as endocardial, myocardial or pericardial, chronic auriculitis and chronic pericarditis are peculiar to the rat. The others have their counterparts in man. With the exception of the infectious processes, almost all the changes appear late in the second year of life and do not attain their maximal incidence until the third year, periods corresponding roughly to middle age and senescence in man—which is a distribution with respect to age analogous to that of many human cardiac conditions. In both species males are somewhat more susceptible to the development of this type of disease than females. Intimal atheromas, such as characterize disease of the human coronary artery, myocardial infarctions, chronic deformities of cardiac valves and rheumatic infections of cardiac valves are not found in the rat. Slight nonspecific inflammatory changes of the mitral valve and of the perivascular tissue of the myocardium occur occasionally.

The manifestations of vascular disease in 487 rats of all ages in which death occurred from natural causes are described. Intimal lesions of the arteries comparable to those of man and birds or to those induced experimentally in rabbits by feeding cholesterol or in coronary arteries of rats by administering excessive doses of vitamin D were not observed. The elastic fibers in the aortas of senile rats were thicker and less undulating than those in the aortas of young ones, which, but for the absence of fraying and splitting, is typical of the human change with age. Only the coronary and pulmonary arteries were commonly the seat of degenerative changes, consisting in fibrosis of the media, thickening of the wall and calcification, particularly in the pulmonary artery. Renal lesions similar to arteriosclerotic atrophy in the human kidney are described, but their association with vascular disease is not established. Generalized arteriosclerosis was not found. The absence of amyloidosis in all the animals is noteworthy. Chronic suppurative lesions were common.

CAROLYN HAMMOND.

DEGENERATIVE ARTHRITIS. G. R. CALLENDER and R. A. KELSER, Am. J. Path. **14**:253, 1938.

Degenerative arthritis is a definite entity, of unknown cause, which commences as a degeneration of joint cartilage and involves the bone only secondarily. It exists in many species of animals and is an important cause of disability in man as well as in horses and mules. The lesions in man and in horses and mules are practically identical in character, though the most advanced lesions are not ordinarily found in equines, since the resulting disability causes these animals to be destroyed before the changes reach such a stage. Though relatively few persons with the disease have been examined, the lesions have not been seen in man prior to the third decade of life, but in persons older than this the incidence increases rapidly and is almost universal when the fifth decade is reached. A relatively small proportion of the lesions give rise to symptoms, but this proportion increases with advancing age as does the extent of the areas involved. Involvement of the car-

tilage alone often appears to be symptomless. When pain occurs, it probably usually results from pressure on subchondral bone which has either been denuded of cartilage or more or less damaged by extension of the degenerative change. In those cases in which loose pieces of cartilage or of bone (joint mice) are present in the joint, pain may be produced as a result of their getting between the joint surfaces. Loss of cartilage substance, causing malocclusion of the joint, is followed by production of bone from the calcified matrix and subchondral bone. This appears to be a compensatory effort to replace the degenerated cartilage with bone and, though secondary, has given the name "hypertrophic arthritis" to the disease. This formation of new bone is rare in man except in the presence of the clinical form of this disease. Symptomatic degenerative arthritis occurs from advanced lesions less often in equines than in man. It appears that the greater the physical activity of the subject, whether man or animal, the more serious does this condition become. While the area of cartilage, the number of joints involved and the incidence of clinical arthritis increase with advancing age, rapid progress of the disease to severe disability appears to be due to other factors, as it may occur in the fifth decade or earlier, as well as in later life. Ankylosis, fibrous or bony, does not occur in this condition, though compensatory formation of bone may be responsible for limitation of joint motion.

From Authors' Summary.

Hypertension in a Patient with a Solitary Ischemic Kidney. Gustave Freeman and George Hartley Jr., J. A. M. A. **111**:1159, 1938.

In a patient in whom high blood pressure developed after removal of a ruptured but otherwise intact kidney, an atheromatous plaque was observed partially occluding the opposite renal artery. Except for terminal pyelonephritis, the remaining kidney was relatively free from vascular, degenerative or inflammatory changes. The situation is analogous to experiments in which hypertension is produced in a dog by partially clamping the artery to one kidney and removing the other kidney. No analogous case was found recorded in the literature.

From Authors' Summary.

Acute Glomerular Nephritis in Rabbits. W. E. Ehrich, R. E. Wolf and G. M. Bartol, J. Exper. Med. **67**:769, 1938.

It has been shown in this paper that in rabbits the structural and functional changes in acute glomerular nephritis produced by nephrotoxins after the method of Masugi are the same as those found in human glomerular nephritis. Anatomically the disease is characterized by proliferation of glomerular cells and in some cases by deposition of fibrin and cresent formation in the glomeruli and by fatty changes in the tubules. The functional changes are: oliguria, proteinuria, hematuria, cylindruria, edema, rise in the blood urea and, according to Masugi and Smadel, rise in the blood pressure, lipuria and fall in the urea clearance and plasma proteins. As there do not appear to be any discrepancies between the experimentally induced disease and human nephritis, the conclusion follows that since the two diseases so closely resemble each other they are probably identical. As to the pathogenesis, it has been shown that the disease begins with a period of latency. This is characterized anatomically by hyperemia of the glomeruli and functionally, in at least a number of cases, by increased diuresis. It follows, therefore, that the theory of Volhard, according to which glomerular nephritis is caused by arteriolar spasms, can no longer be maintained. It has been demonstrated further that the proliferation of glomerular cells is the typical lesion and that the deposition of fibrin and the crescent formation occur only in certain cases, and in these, only in a widely varying number of glomeruli. As crescents are found as early as proliferation, it follows that they should not be regarded as pathognomonic of the subacute phase but as representing a complication which

probably aggravates the disease. As to correlation of morphologic and functional changes, it has been demonstrated that oliguria, marked proteinuria and diminished excretion of cyanol appear at the time when the glomerular changes are at their peak. Evidence has been presented that the oliguria and the decrease in excretion of cyanol in acute glomerular nephritis are chiefly the result of the glomerular damage. It has further been demonstrated that the excretion of azofuchsin is unchanged except for a diminution in the rabbit, which at autopsy shows a marked fatty change of the tubules. The authors regard these observations as evidence that in acute glomerular nephritis in rabbits the glomeruli and tubules may function independently of each other.

FROM AUTHORS' SUMMARY.

GENESIS OF RENAL FIBROSIS. J. B. DUGUID, J. Path. & Bact. **46**:237, 1938.

The development of pathologic fibrosis has been studied in experimental nephritis in rats produced by oral administration of acid sodium phosphate and calciferol. No evidence has been found to support the cellular theory of connective tissue formation or to suggest that proliferation of interstitial cells is an essential factor in renal fibrosis. Renal fibrosis seems to be produced mainly by collapse and condensation of the original interstitial framework of the kidney around atrophic tubules, with subsequent transformation of the reticulum and other tissues into collagen. In some cases an infiltration of a hyaline ground substance into the tissue spaces is seen, and this also appears to form the basis of the formation of the new connective tissue. All the evidence seems to point to renal fibrosis as a degenerative or retrogressive rather than a proliferative phenomenon.

FROM AUTHOR'S CONCLUSIONS.

LYMPHADENOID GOITRE. D. M. VAUX, J. Path. & Bact. **46**:441, 1938.

The relation between Hashimoto's struma lymphomatosa and Riedel's iron-hard goiter is discussed. From a study of 38 cases it is concluded that these conditions are earlier and later stages of the same pathologic process. Histologically in the cases examined, the lesions fall into three stages, early, intermediate and late, which agrees with the clinical findings. The etiologic factors in both conditions are discussed, and it is concluded that lymphadenoid goiter should probably be regarded as an excessive involution of the thyroid following mild thyrotoxicosis.

FROM AUTHOR'S SUMMARY.

STONE FORMATION IN THE JEJUNUM CAUSED BY SURGICAL DRESSING. P. SCHEID, Frankfurt. Ztschr. f. Path. **50**:478, 1937.

Scheid describes a 58 year old woman who presented a tumor in the lower half of the abdomen, marked loss of weight and secondary anemia. At autopsy a fist-sized calculus, in the center of which was a surgical dressing, was found in a markedly dilated jejunal loop. Several fistulous tracts were present between the involved jejunum, other jejunal loops and the transverse colon. The walls of the dilated jejunal loop showed marked muscular hypertrophy, chronic inflammatory changes and a scar involving the whole thickness of the wall. The dressing which caused the formation of the calculus had been left in the abdominal cavity during a cesarean section done about seventeen years prevoiusly. Six years previously the patient was operated on for a herniation in the region of the scar. At that time a tumor in the small intestine was noted but could not be removed because of adhesions. The patient was well until two years before death, when loss of weight, anemia and weakness developed. The stools showed occult blood. The author cites twenty-four cases from the literature, but only in a single instance did the observation resemble his own, and in that instance a much smaller stone was found around the surgical dressing.

ANNEMARIE STRAUSS.

ASCHOFF BODIES IN THE HEART IN TUBERCULOSIS. M. MASUGI, S. MURASAWA and YÄ-SHU, Virchows Arch. f. path. Anat. **299**:426, 1937.

The finding of nodules morphologically similar to or identical with Aschoff bodies in the myocardium of 3 patients dead of tuberculosis, who gave no history of rheumatic disease and in whom no other evidence of this disease was found, led to a systematic histologic study of the myocardium in 215 cases of tuberculosis in which a necropsy was made. In 35 of these, interstitial myocardial lesions similar to Aschoff bodies were discovered. They revealed all gradations from characteristic rheumatic nodules to histologically specific small tuberculous granulomas. There were no other evidences of rheumatic disease in this group. The relatively high frequency of occurrence of noncaseating myocardial granulomas in tuberculosis having been established, the authors next investigated 24 cases of fatal rheumatic pancarditis with Aschoff bodies for the presence of tuberculosis. In 5 there was active tuberculosis and in 11 healed tuberculosis. In the remaining 8 cases there was no macroscopic evidence of tuberculosis, but in 2 of these cases fresh histologic tubercles were found in the crypts of the tonsils. Is the striking association of rheumatic nodules with tuberculosis to be interpreted as a complication of one specific disease, tuberculosis, by another, rheumatism? Or are the Aschoff type of granuloma and the histologically specific tuberculous granuloma both due to the one disease, tuberculosis? The authors can find no support for the former possibility and accept the latter. The production of rheumatic cardiac nodules in one person and of specific tubercles in another speaks for specific differences in the strains of the tubercle bacillus, but the association of both types of lesions in the same person is opposed to such a view and indicates a difference in the host's reactivity to tuberculous infection. Klinge and others have adduced much support in favor of the view that rheumatic disease is a hyperergic manifestation in a person allergic to a specific antigen; the latter, however, may be different in different persons. The authors interpret their findings as indicative of constitutional differences in mesenchymal tissue reactivity and of the possibility of the causation of rheumatism by the tubercle bacillus. O. T. SCHULTZ.

LIPOFUSCIN CONTENT OF VOLUNTARY MUSCLE. W. KNY, Virchows Arch. f. path. Anat. **299**:468, 1937.

The area of pigmentation at the nuclear poles was measured in sections of the sternocleidomastoid, subscapularis, sartorius and gracilis muscles of various age periods. The pigment is present very early in life, reaches a maximum at between 30 and 40 years of age and decreases progressively with advancing years. Its presence and quantity are not associated in any way with atrophy. Its quantity depends on the nutritional state and the functional activity of the muscle, as well as on the age of the subject. O. T. SCHULTZ.

PATHOLOGIC ALTERATIONS IN THE LONG SAPHENOUS VEIN. R. NEUMANN, Virchows Arch. f. path. Anat. **299**:479, 1937.

This is a mathematical investigation of the long saphenous vein. It is based, as the author assures the reader, on the study of 1,380 sections of 61 veins. The veins were removed at varying periods after death from subjects of different age groups. The veins were macroscopically normal, but such factors as general chronic disease, obesity, arteriosclerosis, edema, passive congestion, and thrombosis and varicosity of other veins were taken into account. None of the veins revealed microscopically fatty change or calcification. The chief factor influencing the veins was the nature and condition of the tissues through which the veins ran. The wall of the saphenous vein consists of: (1) an intima composed of endothelium, which is delimited from the rest of the wall by an internal elastic lamella; (2) an inner zone of the media, termed media longitudinalis because the muscle fibers take a spiral longitudinal course; (3) an outer zone of the media, termed media circularis because the muscle fibers take a circular course, and

(4) an adventitia sharply delimited from the surrounding tissue. The mathematics of the investigation consisted in the planimetric determination of the surface area of the lumen and of the wall and of the mathematical relation of lumen and wall to each other, as well as in the determination of the relative thicknesses of the layers of the wall and the relative proportions of smooth muscle, elastic tissue and collagenous tissue. The numerical results were correlated with age and the other factors already noted. For relative increases in the tissue systems of the wall the author has invented the terms "musculosis," "collagenosis" and "elastosis," and for decrease, the word "musculosteresis." The thickness of the wall in relation to the lumen increases with age. Veins with relatively thick walls were observed as a rule in subjects with edema and with varicosities of other veins. Relatively thin-walled veins occurred in those with atherosclerosis and obesity. Walls of average thickness were observed in those with passive congestion or with thrombosis in other veins. Such relationships, however, were not constant.

O. T. SCHULTZ.

CHANGES IN THE SPLEEN IN STASIS OF THE PORTAL SYSTEM. E. JAEGER, Virchows Arch. f. path. Anat. **299**:531, 1937.

In stasis of the portal system and in thrombosis of the splenic or portal vein the degree of splenomegaly is variable and seems to bear no direct relation to the cause of the stasis. Mere mechanical damming back of blood on the spleen does not furnish an adequate explanation of the changes that occur in this organ. In Jaeger's investigation the spleen was perfused with Ringer's solution at body temperature and at an outflow venous pressure of from 10 to 12 cm. of water. It was then perfused with the fixing solution and subjected to histologic study. The effect of venous stasis on the spleen depends on whether the stasis is continuous and uniform. In the stasis characteristic of cardiac decompensation and in that associated with occlusion of the splenic vein, which are constant and uniform, the spleen is only moderately enlarged as the result of engorgement and finally may undergo cyanotic atrophy. In cirrhosis of the liver and thrombosis of the portal vein the stasis is intermittent and varies with waves of hyperemia in the territory drained by the portal system. Such intermittent stasis leads to a work hypertrophy of the spleen, with increase in the size of the venous sinuses and in the thickness of their walls. The splenomegaly associated with thrombosis of the splenic vein is not due to stasis caused by occlusion of the vein. The latter is secondary to hypertrophy of the spleen, caused by a factor, probably inflammatory in nature, acting directly on the spleen. Thrombosis of the splenic and portal veins results from the action of the same inflammatory factor on these veins.

O. T. SCHULTZ.

Microbiology and Parasitology

MACROPHAGE REACTION IN EXPERIMENTAL LOBAR PNEUMONIA OF DOGS. O. H. ROBERTSON and C. G. LOOSLI, J. Exper. Med. **67**:575, 1938.

A study has been made of the macrophage reaction in the pulmonary lesions of dogs killed during the course of experimentally induced pneumococcic lobar pneumonia or dying as a result of the infection. This characteristic transformation of the fixed tissue cells of the lung was found as a constant accompaniment of recovery. It was also present in varying degrees in the great majority of animals that died provided they lived more than forty hours. In general the longer the animal survived the more pronounced the macrophage reaction observed in the lesions of the lungs at autopsy. The numbers of pneumococci in the lesions diminished progressively with the evolution of the cellular change, which terminated in resolution of the pneumonic exudate. Some dogs surviving for four days or more showed practically complete clearing of the pulmonary lesions but succumbed

to overwhelming bacteremia or empyema or both. On the other hand, several animals dying with sterile blood exhibited lesions characterized by little or no macrophage response and the presence of many pneumococci. These findings suggest that recovery from experimental lobar pneumonia in the dog depends on a dual mechanism consisting of a generalized process which prevents or controls invasion of the blood stream and a local one by which the lesion is finally freed from the invading micro-organisms. The nature of each of these processes is discussed.

FROM AUTHORS' SUMMARY.

JAPANESE B ENCEPHALITIS VIRUS: ITS DIFFERENTIATION FROM ST. LOUIS ENCEPHALITIS VIRUS AND RELATIONSHIP TO LOUPING ILL VIRUS. L. T. WEBSTER, J. Exper. Med. **67**:609, 1938.

The virus of the Japanese encephalitis B, obtained from Japanese investigators, has proved virulent for mice and monkeys, which confirms the reports from Japan. It has also been found virulent for monkeys when instilled intranasally and for sheep when introduced intracerebrally or intranasally. This virus is differentiable from St. Louis virus and is similar to the virus of louping ill, according to its reactions in animal species. Serologically, however, it is distinct.

FROM AUTHOR'S CONCLUSIONS.

MEASLES INCLUSION BODIES IN BLOOD AND IN TISSUE CULTURES. J. BROADHURST, G. CAMERON and V. SAURINO, J. Infect. Dis. **62**:6, 1938.

In patients with measles, inclusion bodies are present in the mononucleated white corpuscles of the blood. In tissue cultures, inclusion bodies develop in enormous numbers within the mononucleated white corpuscles and the fibroblasts and as free bodies in the plasma medium. The same types of bodies are present in blood smears of persons suffering from measles and in tissue cultures of the blood of such patients. The greatest difference is that in the tissue cultures there is a greater frequency of denser crescentic and irregular bodies in the affected cells and there are free bodies in the plasma medium. Similar swollen and eruptive disturbances are apparent in the mononucleated white corpuscles in smears and in tissue cultures of blood from patients with measles, the changes in the latter being much more extreme.

FROM AUTHORS' CONCLUSIONS.

TISSUE CULTURES OF HUMAN THROAT INCLUSION BODIES. J. BROADHURST, G. CAMERON and I. TAYLOR, J. Infect. Dis. **62**:21, 1938.

Cytoplasmic inclusion bodies have been cultivated in tissue cultures of various types of cells inoculated with bacteria-free material from the throat of a carrier of such bodies. The cytoplasmic character of these bodies is constant in tissue cultures. Free elementary bodies were not observed in the throat or in the tissue cultures. The bodies thus cultivated vary from extremely minute to larger globular bodies exceeding somewhat in size those found in the epithelial cells of carriers; crescentic bodies (which have not been observed in fresh specimens of material from throats of carriers of the inclusion bodies) have so far been found only in the white blood cell cultures. The affected cells from the throat and in the various types of tissue culture, even when heavily loaded with bodies, show none of the eruptive changes observed in measles blood and measles tissue cultures.

FROM AUTHORS' SUMMARY.

INFLUENZA VIRUS ON THE DEVELOPING EGG. F. M. BURNET and D. LUSH, Brit. J. Exper. Path. **19**:17, 1938.

The results of the authors' experiments are considered, and it is concluded that the swine type antibody in human serum is not specific but is a manifestation of

"generalized" antibody, developing usually about the time of adolescence, as a result of repeated infections with human types of virus.

FROM AUTHORS' SUMMARY.

CONCENTRATION AND PURIFICATION OF BACTERIOPHAGE. J. H. NORTHRUP, J. Gen. Physiol. **21**:335, 1938.

A nucleoprotein isolated from a lysed culture of Staphylococcus is described. The loss in activity when susceptible living or dead bacteria were added to a solution of the protein was proportional to the loss in protein from the solution. Nonsusceptible bacteria removed neither protein nor activity. It is suggested that the formation of phage may be more simply explained by analogy with the autocatalytic formation of pepsin and trypsin than by analogy with the far more complicated system of living organisms. (See also the article "An Ultracentrifugal Analysis of Concentrated Staphylococcus Bacteriophage Preparations" by R. W. G. Wyckoff [*J. Gen. Physiol.* **21**:367, 1938]).

FROM AUTHOR'S SUMMARY.

DISTRIBUTION OF STAPHYLOCOCCI IN RABBITS AFTER INTRAVENOUS INOCULATION. J. FORSSMAN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **91**:165, 1937.

Rabbits were inoculated with virulent strains of staphylococci in doses that were fatal in from seventeen to twenty-four hours. The animals were killed at frequent intervals. Cultures were taken from different organs, and microscopic sections of tissue were stained for staphylococci. Large numbers of these organisms were found in the spleen, liver and bone marrow during the first few hours after inoculation. They decreased rapidly, so that very few were found after from six to nine hours. In the heart and kidneys the numbers of the organisms were very small at first, but toward the end the numbers in these two organs were very large. Relatively small numbers of staphylococci were detected in the lungs and muscles during the entire course. The lesions in the heart and kidneys were very similar to those in man in staphylococcic infections.

I. DAVIDSOHN.

RELATIONS BETWEEN THE VIRUS OF PEMPHIGUS AND THAT OF RABIES. E. SCHWEINBURG and S. WOLFRAM, Ztschr. f. Immunitätsforsch. u. exper. Therap. **91**:341, 1937.

The serum of patients with pemphigus and that of rabbits that had been immunized with the virus of pemphigus were mixed with fixed virus and kept in the ice box for twenty-four hours, after which the mixtures were inoculated intracerebrally into guinea pigs. A large majority of the animals remained alive, as compared with the control animals, which had been inoculated with mixtures of fixed virus and normal rabbit or human serum or saline solution. Rabic immune serum mixed with the virus of pemphigus was kept at 0 C. for twenty-four hours and then injected subdurally into rabbits. Also here marked protection was observed. Rabbits immunized against pemphigus showed only slight resistance against rabies, while those immunized against rabies were protected against the virus of pemphigus to a higher degree.

I. DAVIDSOHN.

Immunology

SIGNIFICANCE OF SOME EFFECTS OF FORMALDEHYDE FOR THE THEORY OF ANTIGEN-ANTIBODY AGGREGATION. H. EAGLE, J. Exper. Med. **67**:495, 1938.

The present experiments do not support the theory that antigen-antibody aggregates are lattice-like structures built up from elementary antigen-antibody com-

pounds because of residual specific combining groups. The aggregating activity of both antipneumococcus serum and diphtheria antitoxin was completely inhibited by procedures which did not demonstrably affect their power of combining with antigen. This suggests that the aggregation of antigen-antibody compounds is a secondary, nonspecific reaction. It is perhaps significant that the amount of formaldehyde which just sufficed to prevent aggregation also caused a marked increase in the solubility of the pneumococcus antibody, which could then no longer be precipitated at serum p_H by dilution with water or by dialysis. This strongly suggests that the loss of precipitating activity is actually due to the increased solubility of the antibody and supports the hypothesis that the primary cause of specific antigen-antibody aggregation is the relative insolubility of the bound antibody.

FROM AUTHOR'S SUMMARY.

LOCAL RECOVERY IN EXPERIMENTAL PNEUMOCOCCIC LOBAR PNEUMONIA IN THE DOG. O. H. ROBERTSON and L. T. COGGESHALL, J. Exper. Med. **67**:597, 1938.

An investigation has been made of the process of local recovery occurring in the pulmonary lesions of dogs with experimental pneumococcic lobar pneumonia. Six animals showing simultaneous healing and spread of the pathologic process in different parts of the lungs were put to death during the active state of the disease for bacteriologic and histologic study. It was found that with few exceptions the lesions that were clearing (resolving), as revealed by roentgenograms taken during life, were sterile on culture, while the young, metastatic processes yielded an abundant growth of pneumococci. The resolving areas, which represented the older lesions, were characterized by the presence of a well developed macrophage reaction, whereas the early lesions consisted of alternating areas of edema and polymorphonuclear infiltration and contained usually many pneumococci, both free and in the cells. The only pneumococci found in the areas of mobilization of macrophages were intracellular forms, for the most part in the process of digestion. In several instances these striking differences in the cellular picture and in the numbers and distribution of pneumococci were observed in different parts of a single lobar lesion. Tests on the blood serum failed to reveal any evidence of acquired antipneumococcic humoral immunity. The significance of these findings in relation to the mechanism of recovery is discussed.

FROM AUTHORS' SUMMARY.

A STABLE HEMOLYSIN-LEUCOCIDIN AND ITS CRYSTALLINE DERIVATIVE ISOLATED FROM BETA HEMOLYTIC STREPTOCOCCI. E. J. CZARNETZKY, I. M. MORGAN and S. MUDD, J. Exper. Med. **67**:643, 1938.

A chemically pure hemolysin-leukocidin has been isolated from hemolytic streptococci of the beta type but not from other species of bacteria studied. It does not give rise to antibodies but precipitates immune serum against hemolytic streptococci, and is therefore a hapten. A highly purified sample in dilutions up to 1:128,000 hemolyzes red blood cells. Its hemolytic activity is not spefiically neutralized by antiserum against beta hemolytic streptococci. It is leukocidic in that it inhibits the reduction of methylthionine chloride by leukocytes. The hemolysin-leukocidin is stable to oxygen, to heat and to moderate changes in hydrogen ion concentration. Its chemical structure has been determined in part. Its molecular weight is 2,260. A crystalline derivative has been isolated as the sodium salt from the hemolysin-leukocidin. As the free acid it has a molecular weight of 720. Its hemolytic and leukocidic activity parallels that of the highly purified stable hemolysin-leukocidin, although it is not serologically active. It possesses a high degree of toxicity for mice and rabbits.

FROM AUTHORS' SUMMARY.

MENINGOCOCCAL BROTH CULTURE FILTRATES: FAILURE OF PROTECTION EXPERIMENTS. B. G. MAEGRAITH, Brit. J. Exper. Path. **19**:95, 1938.

The experiments outlined demonstrate that the "antitoxic" antibacterial and normal serums examined have no power of neutralizing the poisonous filtrates of broth cultures of meningococci. Serums prepared by injection of these filtrates into rabbits and horses have low agglutinating and precipitating properties and are devoid of protective action against the filtrates. These results are in agreement with those obtained by other workers referred to, so that there appears to be no reliable experimental evidence at present in support of the use of such toxic filtrates for the preparation of therapeutic serum.

FROM AUTHOR'S SUMMARY.

ELIMINATION OF BLOOD GROUP SUBSTANCES. V. FRIEDENREICH, Ztschr. f. Immunitätsforsch. u. exper. Therap. **91**:39, 1937.

It was shown that secretions (saliva, sperm, etc.) of some persons (about 70 per cent) possess the same group properties as their red blood cells, while the secretions of the other 30 per cent lack the group properties. Friedenreich finds that Schiff's original explanation in which he suggested that there is a secretion of circulating blood group properties does not withstand critical analysis and suggests another hypothesis: a production of the group properties in the parenchyma of the secreting glands of some persons and a lack of production in the glands of others. This is corroborated by the finding of the A property in the saliva of some horses and the failure to find it in other horses. The A property could not be demonstrated in the blood of any of the horses.

I. DAVIDSOHN.

HEMAGGLUTINATION IN OWLS (STRIGIDAE). P. DAHR, Ztschr. f. Immunitätsforsch. u. exper. Therap. **91**:97, 1937.

Isoagglutination was not detected in cross agglutination experiments between the bloods of 27 members of this family. Some of them had antihuman species agglutinins. A few had group specific anti-A and anti-B agglutinins. Three had anti-O agglutinins. The properties A, B, M and N were not found.

I. DAVIDSOHN.

AUTOAGGLUTINATION. E. POULSEN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **91**.:135, 1937.

While Neuda reported autoagglutination in the serum of almost all patients with cancer, Poulsen found it in the serum of only 1 of 20 patients. Inactivation of serum at 56 C. weakened only slightly its ability to remove the corresponding isoagglutinins from other serums. Some liver extracts were shown to possess property A.

I. DAVIDSOHN.

PURIFIED HEMAGGLUTININS. P. DAHR, Ztschr. f. Immunitätsforsch. u. exper. Therap. **91**:149, 1937.

To meet the technical difficulties inherent in the preparation of solutions of purified hemagglutinins according to Landsteiner, Dahr places the saline suspension of the sensitized and washed red blood cells in a small test tube, then puts this tube into a centrifuge tube and surrounds the inner tube with liquid paraffin. A properly constructed stopper closes both tubes. The two tubes are placed for fifteen minutes into a water bath at 55 C. The temperature is maintained during the subsequent centrifuging.

I. DAVIDSOHN.

AGGLUTINOGEN B IN ANTHROPOID AND LOWER APES AND IN OTHER MAMMALS. P. DAHR, Ztschr. f. Immunitätsforsch. u. exper. Therap. **91**:211, 1937.

The agglutinogen B consists of three fractions—B_1, B_2 and B_3. All of these are present in man and in anthropoid apes, while B_2 or B_3 or both may be found in some of the other animal species, but not B_1. Human anti-B serums (from groups A and O) contain similar fractions in the isoagglutinins; the anti-B_1 fraction is apparently rare. With the use of proper serums, Dahr found the B fractions distributed as follows: B_1, B_2 and B_3 in an orang-utan, B_2 and B_3 in the rabbit, kangaroo, cat, tapir and marten and B_3 in the guinea pig, capuchin monkey, African and Indian elephants, dog, badger and bison. Neither agglutinogen A nor agglutinogen B was found in birds. The distribution of factor B seemed independent of the position of the species on the evolutionary scale.

I. DAVIDSOHN.

INVESTIGATIONS ON THE M-N, A_1-A_2 AND O-A-B BLOOD GROUPS IN FINLAND. E. MUSTAKALLIO, Acta Soc. med. fenn. duodecim (Ser. A., no. 2, art. 4) **20**:1, 1937.

Bloods of 2,595 healthy persons and 4,525 sick persons were studied. The results of the blood-grouping tests on the combined series (7,120 persons) were as follows: group O, 33.9 per cent; group A, 43 per cent; group B, 15.8 per cent; group AB, 7.3 per cent. Most of the persons of group A and group AB were tested in order to ascertain which of the two subgroups they belonged to, the observed frequencies being as follows: A_1, 32.3 per cent; A_2, 10.7 per cent; A_1B, 4.4 per cent, and A_2B, 2.9 per cent. In addition, 6,813 of the bloods were classified according to their content of M-N agglutinogens, and it was found that 39.5 per cent contained type M, 13.7 per cent type N and 46.8 per cent type MN. These figures are interesting because of the high incidence of type M and the low incidence of type N, so that with regard to these properties the Finns lie between the various European races and the American Indian. No difference in the distribution of the blood groups, subgroups or M-N types was found among persons suffering from a large variety of diseases in comparison with the distribution among normal persons, nor was there any variation among different age groups or between the two sexes. The inheritance of the blood groups, the M-N types and the subgroups of group A was studied in 29 families with 72 children, also among 44 mothers and their 115 children. No exceptions to the established theories of heredity was encountered.

A. S. WIENER.

Tumors

NEUROFIBROMATOSIS. O. A. TURNER and W. J. GARDNER, Am. J. Cancer **32**:339, 1938.

A family has been described in which one or more tumors of the sheaths and enveloping membranes of the nervous system appeared as a hereditary trait, transmitted as a mendelian dominant. The incidence of this condition in 6 members of this family is reported in detail. Of the 14 members of the family, 7 gave evidence of having been affected. In the latter group, 3 were proved at autopsy and 2 were proved clinically to have cutaneous nodules typical of von Recklinghausen's disease. One member of the family had clinical signs which were regarded as probably due to early formation of an intracranial tumor, and the family history indicated that another member had peripheral and possibly central neurofibromatosis at the time of death. It has been suggested that von Recklinghausen's disease be regarded as a process involving all the sheaths and enveloping membranes of the nervous system and that this concept in a broad sense be extended to include the glial elements as well. Proof that the defect is in the binding tissues of the nervous system would account not only for the various types of growths associated with central neurofibromatosis but also for the preponderance of one type over another.

FROM AUTHORS' SUMMARY.

INTRANUCLEAR BODIES IN NORMAL AND NEOPLASTIC HUMAN TISSUE. R. C. PAGE, J. F. REGAN and W. C. MACCARTY, Am. J. Cancer **32**:383, 1938.

Intranucleolar bodies have been found in cells of normal tissues and in those of benign and malignant tumors by the use of general and specific stains. The intranuclear bodies that appear as unstained areas or vacuoles are called "argentophile bodies." Refractive bodies are best seen in fresh frozen sections stained with polychrome methylene blue. Refractive bodies are greater in number and larger in cells of benign and malignant tumors than they are in normal cells. Argentophilic bodies are present in greater numbers in cells of benign and malignant tumors than they are in normal cells. The more malignant the neoplasm, the greater is the number of intranucleolar bodies. FROM AUTHORS' SUMMARY.

AGGLUTINATION TESTS IN THE STUDY OF TUMOR IMMUNITY, NATURAL AND ACQUIRED. T. LUMSDEN, Am. J. Cancer **32**:395, 1938.

The natural resistance of any rat to implantation of a tumor is directly proportional to its capacity to produce agglutinins and chromatolysins and is inversely proportional to the amount of corresponding agglutinogens in its body cells, of which those in its blood corpuscles act as an approximate index. It follows that, by estimating these agglutinogens by means of the strongly agglutinating serum of rats immune to Jensen sarcoma, it is possible to select certain rats in which a tumor graft is sure to regress and with somewhat less accuracy others in which it will grow progressively. FROM AUTHOR'S SUMMARY.

SPONGIOBLASTOMA UNIPOLARE. D. H. ECHOLS, Arch. Neurol. & Psychiat. **39**:494, 1938.

In a series of 263 cases of cerebral glioma, Echols found 12 instances of spongioblastoma unipolare. It is a slowly growing benign glioma, and in the author's cases it affected the optic chiasm, optic nerve, pons, medulla, cerebral and cerebellar hemispheres and acoustic nerve. It consists of unipolar or bipolar spongioblasts, piriform or spindle shaped, with a long process extending from one or both poles and usually heavily stained. The average patient's age on admission was about 21 years. The tumor can be recognized clinically. G. B. HASSIN.

INFLUENCE OF TEMPERATURE ON PROLIFERATION OF INFECTIOUS FIBROMA AND INFECTIOUS MYXOMA VIRUSES IN VIVO. R. L. THOMPSON, J. Infect. Dis. **62**:307, 1938.

Increasing the temperature of the skin of the rabbit to a value approximating that of deeper tissues in the body inhibits the development of lesions in this tissue by the virus of fibroma. This observation is interpreted as indicating that this virus is unable to proliferate at the ordinary temperature of the body and consequently the observation may partially explain why this virus normally parasitizes the superficial tissues of the body.

The virus of myxoma, which is antigenically related to that of fibroma, is definitely influenced but to a less extent by fever therapy.

Immunity was not observed in those animals which failed to show evidence of active infection. This was noted with both viruses.

FROM AUTHOR'S SUMMARY.

INFLUENCE OF NONBREEDING AND FOSTER NURSING ON THE OCCURRENCE OF SPONTANEOUS BREAST TUMORS IN STRAIN C$_3$H MICE. H. B. ANDERVONT, Pub. Health Rep. **53**:777, 1938.

Breeding females of strain C$_3$H have a high incidence of spontaneous tumor of the breast at an average age of from 8 to 9 months. Nonbreeding females have

the same high incidence, but at an average age of 11.5 months. It is concluded that pregnancy hastens the appearance of spontaneous tumor of the mammary gland in this colony of strain C3H mice.

The preliminary findings in a foster nursing experiment in which strain C3H mice were nursed by C57 black mice show that up to the age of 13.5 months strain C3H mice exhibit an incidence of tumor of approximately 21.5 per cent, which is much lower than the incidence in breeding or nonbreeding mice of the same age. It is concluded that foster nursing by C57 black mice exerts a decided influence on the occurrence of spontaneous mammary tumors in strain C3H mice.

FROM AUTHOR'S SUMMARY.

BONE METASTASES OF TRANSPLANTABLE TUMORS. W. SCHOPPER, Virchows Arch. f. path. Anat. **298**:527, 1937.

The Brown-Pearce rabbit carcinoma differs from most transplantable tumors in its greater tendency to produce distant metastases, especially in bones. When this growth is inoculated intravenously, the incidence of bone metastases is greatly increased. The bodies of the vertebrae are frequently involved, but the growths are microscopic and evoke little reaction on the part of the vertebrae. In the lower end of the femur and the upper end of the tibia gross tumors are produced, which are both periosteal and endosteal. In carcinomatous metastasis to bone in man, periosteal involvement is usually held to be secondary to involvement of the marrow. In the rabbit the periosteal metastasis may arise independently of the endosteal. In the marrow the tumor destroys the osseous trabeculae. In the periosteum it evokes considerable osteoblastic new formation of bone, although there is also some destruction of bone. Intravenous inoculation of the Brown-Pearce carcinoma usually leads to death of the animals in from two to four weeks.

O. T. SCHULTZ.

SPLENOMA. E. MORDASINI, Virchows Arch. f. path. Anat. **298**:594, 1937.

Under the term "splenoma" Mordasini describes 6 cases of tumor-like nodules of the spleen. The nodules occurred in persons whose ages varied from 23 to 84 years. Four of the patients were men. Two were women. Nodules of this type may be solitary or multiple. They may vary in size from those just visible to others as large as the fist; the largest described by the author had a maximal diameter of 11 cm. and was larger than the spleen to which it was attached by a pedicle. In 2 cases the condition was considered to be hamartia (Albrecht), i. e., a maldevelopment of tissues normal for the situation such that these tissues reveal a quantitative abnormal relationship to each other. In the remaining cases the splenomas were hamartomas (Albrecht). In the hamartoma the quantitatively predominating element has the property of independent and continued growth. Some of the tumors have a lymphoid follicular structure; others are composed of splenic pulp. They may undergo fibrosis as the result of passive congestion or hemorrhage. The smaller nodules are usually situated immediately beneath the capsule. They undergo the same evolutionary and involutionary changes as the spleen in which they occur. They cause no clinical symptoms and no changes in the blood picture.

O. T. SCHULTZ.

BILATERAL TUBULAR TESTICULAR ADENOMA ASSOCIATED WITH INTERSEXUALITY. I. KRÜCKMANN, Virchows Arch. f. path. Anat. **298**:619, 1937.

Nineteen previously reported cases of tubular testicular adenoma are briefly summarized and are placed in two main groups, each with two subgroups. Group 1 consists of cases in which the tumor developed in the ectopic testis of (*a*) a male with no other apparent anomaly and (*b*) a pseudohermaphroditic male. Group 2 consists of cases in which a testicular adenoma developed in the ovary of (*a*) a woman with no other abnormality than menstrual disturbance and (*b*) a

woman with pseudohermaphrodism. The case reported in detail was that of a 25 year old person with a feminine habitus of body, well developed mammary glands and external genitalia of the female type but with no vagina. Death followed the second stage of an operation undertaken to provide a vagina. At necropsy no vestige of female internal genitalia could be discovered. Each of the two ectopic testes was the seat of a tubular adenoma.

O. T. SCHULTZ.

CYSTIC DISEASE OF THE BREAST AND CARCINOMA. H. BERNING and J. BÜCKER, Virchows Arch. f. path. Anat. **298**:728, 1937.

The possible relation of carcinoma of the mammary gland to cystic disease, for which the authors prefer the term "mastopathia cystica," is discussed in relation to the presence of ducts or cysts lined by epithelium like that of the apocrine sweat glands of the axilla. Statistical correlation of the observations on 2,283 specimens of mammary tissues examined histologically by Schridde, from whose institute this study comes, leads to the conclusion that the occurrence of carcinoma and cystic disease in the same breast is a matter of coincidence. Some have held that glands of the apocrine type occur in the breast as the result of metaplasia and represent a precancerous stage of mammary carcinoma. Berning and Bücker examined microscopically mammary tissues removed at necropsy from 50 women between the ages of 16 and 70 years who had shown no evidence of either cystic disease or carcinoma. In the tissues from 42 of these women apocrine glands were found, and the authors believe that sufficiently prolonged search might reveal their presence in all normal breasts. They hold that the apocrine glands arise as the result not of metaplasia but of heteroplasia, that this process may begin even before birth, that the cells are fully differentiated and reveal no tendency to anaplasia, that like other heteroplastic tissues the apocrine glands of the breast reveal no tendency toward blastomatous proliferation, that they bear no relation to carcinoma and that their presence is not indicative of a precancerous state.

O. T. SCHULTZ.

ELEMENTARY (PASCHEN) BODIES IN INFECTIOUS MYXOMA OF THE RABBIT. C. E. VAN ROOYEN, Zentralbl. f. Bakt. (Abt. 1) **139**:130, 1937.

Typical elementary bodies, morphologically similar to the elementary, or Paschen, bodies of vaccinia, were demonstrated in infectious myxoma of the rabbit. They varied in size from 0.31 to 0.36 micron, whereas the elementary bodies of vaccinia, when measured under identical conditions, varied from 0.25 to 0.30 micron in diameter. The elementary bodies of myxomatosis also appeared in greater numbers and occasionally showed evidence of a formation of short chains. Experiments on rabbits immunized against the virus of vaccinia, as well as tests on animals of seven different species, proved that the strain of myxoma virus used for study was free from contamination with vaccinia virus.

PAUL R. CANNON.

Technical

THE WELTMANN SERUM COAGULATION REACTION. S. A. LEVINSON and R. I. KLEIN, Am. Rev. Tuberc. **37**:200, 1938.

The Weltmann serum coagulation reaction (*Med. Klin.* **26**:240, 1930) is of importance in distinguishing between exudative and productive changes. In general, acute inflammatory and exudative conditions give a shortened coagulation band or a shift to the left. Chronic diseases (characterized by fibrotic changes), the healing stage of acute infection and damage of the parenchyma of the liver give a lengthened coagulation band or a shift to the right. The coagulation band does not always parallel the sedimentation rate. The Weltmann coagulation test can be used as a guide in determining the course and outcome of disease, and it reflects tissue changes occurring in the body. When exudative and fibrotic

changes occur in the lung at the same time, the Weltmann reaction may not be of great assistance, as the coagulation band may strike the normal zone. In miliary tuberculosis, peritoneal tuberculosis and meningeal tuberculosis there is a shortening of the band in the exudative zone. Isolated tuberculosis of bone is characterized by a normal or prolonged band (fibrotic zone). There is a correlation between the concentration of hydrogen ions in the blood and Weltmann reaction which is believed to be directly related to the underlying process. Although it is not possible at present to explain the mechanism of the reaction, the latter may be of interest in the diagnosis and prognosis of disease, particularly tuberculosis.

H. J. CORPER.

ADAPTATION OF THE ORIGINAL WEIGERT TECHNIC FOR STAINING MYELIN SHEATHS IN FORMALDEHYDE-PYROXYLIN MATERIAL. D. M. SCHWAB and T. J. PUTNAM, Arch. Neurol. & Psychiat. **38**:1291, 1937.

Sections from blocks embedded in pyroxylin may be stained for the demonstration of axis-cylinders, glia, blood vessels and ganglion cells by well known modified staining methods (Bielschowsky, Alzheimer-Mann, Cajal, etc.). The authors add a modified Weigert method which can be used successfully on sections from blocks embedded in pyroxylin. The sections, cut from 25 to 30 microns thick, are dehydrated in graded alcohols, cleaned of pyroxylin by equal parts of alcohol and ether, by methyl alcohol or by acetone and carried through absolute alcohol, 95 per cent alcohol and so on to distilled water. The sections are then placed in Weigert's rapid mordanting fluid (chromium fluoride, formaldehyde and copper acetate) for from twenty-four to forty-eight hours at 37 C.; they are then washed in distilled water and stained in a 10 per cent alcoholic solution of hematoxylin of which 10 cc. has been diluted in 2 per cent acetic acid. Here they are kept for from twelve to twenty-four hours at 37 C.; they are then washed in distilled water, carried through Müller's fluid (an aqueous solution of 2.5 per cent potassium dichromate and 1 per cent sulfate), differentiated according to the Weigert-Pal method, washed in distilled and tap water, dehydrated, cleared with xylene and mounted in balsam. This method is not satisfactory for sections embedded in paraffin.

GEORGE B. HASSIN.

TRANSFUSION SYPHILIS. C. R. REIN and others, J. A. M. A. **110**:13, 1938.

The responsibility of preventing transfusion syphilis rests with the physicians of hospitals and other institutions, both public and private, dedicated to the care of the sick. The control of donors under municipal supervision should be rigidly enforced.

With the adoption of the practice of carrying out blood tests on all donors immediately prior to transfusion, the periodic testing of the blood at predetermined intervals would become unnecessary. Even if professional donors are subjected to blood tests at monthly intervals, accidents cannot be wholly eliminated, for the danger of infection of the donor in the intervals between tests is a more than theoretic possibility.

A thoroughly reliable, rapid and easily performed blood test, if done on every donor immediately prior to transfusion, would practically eliminate all risk of infection of the recipient and would give the donor a clean bill of health at a time when serologic control is of paramount importance.

The Kline flocculation test embodies the desirable features of such serologic control because of ease and rapidity of performance, adequate specificity and high degree of sensitivity.

FROM AUTHORS' SUMMARY.

Society Transactions

CHICAGO PATHOLOGICAL SOCIETY

KATHARINE M. HOWELL, *President*

Regular Monthly Meeting, Oct. 10, 1938

EDWIN F. HIRSCH, *Secretary*

PRESIDENTIAL ADDRESS: INCIDENCE OF AMEBIASIS, TYPHOID AND DYSENTERY IN A CHICAGO HOSPITAL. KATHARINE M. HOWELL.

In May 1936 a transitory epidemic of mild diarrhea occurred in the Michael Reese Hospital, limited apparently to the hospital personnel. The diarrheal stools were examined, and several were found to contain Endamoeba histolytica. Although E. histolytica was obviously not the cause of this brief epidemic of diarrhea, it was deemed advisable to determine the incidence of amebiasis in the hospital personnel. The stools were examined for typhoid, paratyphoid, dysentery and other intestinal organisms, as well as for amebas. The bacterial cultures were included with the object of diagnosing the occasional bacillary diarrhea, finding any carriers of these organisms in the personnel of the hospital and recognizing mixed infections of E. histolytica and bacillary organisms.

The survey was started in May 1936. The parasitologist, Elta Williams Knoll, was experienced in the diagnosis of amebas; she had been one of the workers requested to take part in the 1933 survey of the Chicago Board of Health. Several additional experienced technicians were secured to facilitate the survey. The personnel of all departments in contact with patients, such as food handlers, nurses, laboratory workers and others, submitted feces for examination. Other employees who wished to do so also sent samples. The technic employed in examining the stools for E. histolytica was essentially that established by the Chicago Board of Health in 1933; it consisted in examining two moist smears and culturing suspected feces in Cleveland's medium (*Am. J. Hyg.* **12**:606, 1930). If the specimen of feces in any way suggested the presence of E. histolytica, another sample was requested. Subsequent surveys indicated that a high percentage of the persons who were infected had been detected in the initial survey. Identical specimens of feces were cultured for bacteria, standard methods being used. Cultural identification was confirmed by agglutination tests with known antiserums.

The physical construction of the hospital was thoroughly examined and found to conform in all respects with regulations made by the Chicago Board of Health in 1933 as to plumbing, drinking water and other details. The water supply was filtered through sand and paper, a method found efficient for the removal of amebic cysts (Spector, B. K.: *Pub. Health Rep.* **51**:1567, 1936). The monthly bacterial examination of the water supplies revealed no contamination with sewage. Many samples of food, milk, fresh fruits and vegetables were examined for E. histolytica, with negative results. The nursing technic was inspected, and the importance of personal hygiene was impressed on all employees. No foci for the dissemination of E. histolytica or bacillary organisms were found. It seemed probable, therefore, that amebiasis in the hospital was a matter of carriers.

The first survey of the personnel of the hospital for infection with E. histolytica was completed in September 1936. Of the 1,287 specimens examined, 113 (9 per cent) contained some form of E. histolytica. The percentage was considerably higher than that found among food handlers in 1933 (2.5). The persons harboring

E. histolytica received treatment for amebiasis, and there was immediately a 50 per cent decline in the incidence. These carriers were subsequently examined at frequent intervals.

The investigation was continued in order to eradicate clinical and carrier amebiasis from the personnel of the hospital. The following procedure was instituted: All applicants for positions in the hospital were examined for infection with E. histolytica and for infection with typhoid-dysentery organisms. Since the examinations were started in 1936 the incidence of amebiasis in these applicants has been consistently between 9 and 10 per cent. During 1936 the entire personnel of the dietary department was examined three times, and since then, twice a year. Ward helpers, maids and orderlies have been examined once a year. Interns and laboratory staff were examined in 1936. A part of the initial physical examination of a new member is an examination of the stools. In the initial survey the entire personnel in the nursing department was examined for infection with E. histolytica. Since 1936 the incoming classes, affiliates from other hospitals and all persons previously found infested have been examined for amebiasis.

The nurses as a group were chosen for questioning with regard to symptoms. Many admitted that there had been occasional gastrointestinal disturbances, intermittent diarrhea and constipation, vague intestinal symptoms and a feeling of lassitude, but such complaints may be elicited both in those who are infected and in those who are not infected with E. histolytica. Many persons whose stools were grossly characteristic of amebiasis and contained trophozoites or cysts denied having any symptoms. After treatment for amebiasis, however, some of these persons volunteered the information that they felt in better health than formerly. The incidence of infection with amebiasis was twice as high among the nurses whose homes were in Chicago or who were in training in Chicago during the epidemic of 1933 than in the nurses from outside Chicago. During the time in which the personnel was examined, the incidence of infection with E. histolytica among patients was observed. Specimens of stools were not solicited for examination, but those sent to the laboratory to be cultured for bacteria or examined for parasites were studied. The incidence of amebiasis was low.

Typhoid and Dysentery in the Hospital.—Typhoid and paratyphoid bacilli were not found among the personnel. One acute infection with Bacillus paratyphosus B was present. One person harbored Bacillus dysenteriae Flexner and cysts of E. histolytica. She was apparently in good health and stated that she had not noted any gastrointestinal disturbances. There were 4 cases of bacillary dysentery, 1 of the Shiga type and 3 of the Flexner. Following these acute infections there was no increase in the incidence of diarrhea in the hospital personnel. No other intestinal organisms were isolated from stools that could be associated consistently with mild attacks of diarrhea. Apparently, there was no carrier of typhoid or paratyphoid bacilli among the personnel, owing probably to the initial examinations of stools and to the routine immunization against typhoid and paratyphoid bacilli. The incidence of infection with typhoid and dysentery organisms was higher in the patients than in the hospital personnel. There were 11 cases of typhoid and paratyphoid and 26 cases of bacillary dysentery among the patients at Michael Reese Hospital during the two and a half year's observation.

Observations.—It was impossible to classify persons as carriers or as subjects of active amebiasis according to whether the stool contained cysts, precysts or trophozoites, because at times all or two stages were found in the same specimen of feces. However, stools from persons with active amebiasis usually contained trophozoites, and those from persons who were almost symptomless contained cysts.

Different races of endamebas were noted throughout the investigation. Ninety per cent of the cysts were from 12 to 14 microns in diameter, the type usually contained in stools. About 5 per cent were small, from 5 to 8 microns in diameter, and about 5 per cent were large, from 18 to 20 microns in diameter. No correlation of clinical findings was noted with the variety of E. histolytica observed.

E. histolytica was almost always associated with other amebas or other parasites. This was such a common finding that if other parasites were present and E. histolytica was not observed, a request was made for repeated specimens of stools in order that a further study might be made. Little association was found between infection with E. histolytica and infection with bacteria of the typhoid and dysentery groups, probably because such bacterial infection among the personnel was uncommon. One employee was apparently a healthy carrier of cysts of E. histolytica and of the Flexner bacillus. One patient with acute dysentery had both trophozoites and dysentery bacilli of the Flexner type in repeated specimens of stool. Most persons after a course of treatment for amebiasis were entirely free from cysts or vegetative forms of E. histolytica, as well as from clinical symptoms. There have been a few recurrences of amebiasis or possibly reinfections with E. histolytica.

Comment.—From May 1936 to May 1937, 1,581 examinations for infection with E. histolytica were made on the personnel of Michael Reese Hospital; 130, or 8.2 per cent, showed infection. Of the 3,220 examinations made on both personnel and patients, 165, or 5.1 per cent, revealed E. histolytica. In 1937-1938, 3.6 per cent of the personnel and 1.97 per cent of the combined groups were shown to harbor E. histolytica. Several other hospitals in Chicago made surveys for this organism in 1936. Spector (*Am. J. Pub. Health* **27**:694, 1937) examined several groups at the University of Chicago Clinic, obtaining positive results in such percentages as 10, 11 and 8.3. McDaniels, Burton and Arnold (*Am. J. Digest. Dis. & Nutrition* **3**:526, 1936) obtained positive results in 6.3 per cent at the Educational and Research Hospital of the University of Illinois, and in 8.9 per cent of the personnel of the Chicago Memorial Hospital. There was only slight variation in the incidence of infection with E. histolytica as disclosed in these surveys made on similar groups in the same locality and by fairly standardized methods of examination. With one factor changed, the group, Spector found the percentage of persons harboring E. histolytica considerably higher—for example, 17 per cent among firemen and 21 per cent among men in shelters. These results correspond to Craig's statement that amebiasis is endemic throughout the United States and that the incidence is approximately 10 per cent of the population (*Am. J. Pub. Health* **28**:187, 1938).

Summary.—In Michael Reese Hospital amebiasis is a matter of carriers. The number of carriers among the personnel has been reduced 50 per cent. The incidence of amebiasis in the hospital in 1936 was comparable to that in similar groups in Chicago. The incidence of amebiasis in the patients is low. The incidence of typhoid, paratyphoid and dysentery is low both in the personnel and in the patients.

PERIARTERITIS NODOSA. NATHAN B. FRIEDMAN.

A boilermaker aged 59 years had an unexplained fever of about two months' duration. He complained of severe pain in the lower extremities. The disease had a septic course, and there was leukocytosis. Cultural and agglutinative studies of the blood and excreta disclosed nothing of importance. At times pain in one testicle, edema of one foot, abdominal distention, rigidity and melena were noted.

Autopsy disclosed widespread periarteritis nodosa with involvement of the vessels of nearly every organ except the brain. The microscopic structure was that of acute, organizing and healed thromboarteritis and periarteritis. Acute and healed infarcts were present throughout the viscera. In the appendix the process had led to perforation with localized peritonitis. A large tumor in the spleen had metastasized to the regional lymph nodes. Microscopically, it was an undifferentiated reticulum cell sarcoma. An additional condition was nodular pneumonoconiosis of undetermined type.

CARCINOMA OF THE THYROID WITH A LONG CLINICAL HISTORY. JOSEPH L. MILLER JR.

Both patients were men. When the histologic diagnosis was made, one was 39 and the other 27 years old. Each was alive at least six and a half years after metastatic carcinoma had been diagnosed on the basis of histologic examination. At the end of the six and a half years one patient died, The anatomic diagnosis at autopsy was carcinoma of the thyroid gland with metastases. The main tumor mass in the neck measured 20 by 12 by 5 cm. At the end of the six and a half years the entire thyroid gland of the other patient was removed. In one lobe a tumor, 1 cm. in diameter, was found, presumably the primary carcinoma. The treatment prior to the end of the six and a half year period consisted, in one case, of incomplete removal of the palpable nodules in the neck and roentgen irradiation of the regional tissues and, in the other case, of removal of nodules in the neck diagnosed as metastases. Clinically the two patients did not present evidence of hyperthyroidism. Both had diarrhea of undetermined cause. The first specimen of tumor obtained from the elder patient showed solid masses and strands of cells but no acini. The specimens of tumor obtained at autopsy in this case, including a metastasis removed at that time, had large follicle-like structures, some of which contained a hyaline material. The sections of tumor tissue from the younger patient all showed masses of cells with some tendency toward the formation of acini. Tissues from both patients showed occasional intercellular aggregations of hyaline or amorphous material. The first tissue removed from the elder patient conformed to some extent to the group which Smith, Pool and Olcott called the fetal type of adenocarcinoma (*Am. J. Cancer* **20**:1, 1934). These investigators said that tumors of this group are characterized by low to moderate malignancy and may tend to have a solid structure without acini.

DISCUSSION

H. G. WELLS: There has been some discussion in the German literature about apparently benign thyroid tissues giving rise to metastases and the question arose whether they should be termed carcinomas or metastasizing thyroid glands. Were there many cells in mitosis in your tumors?

I. DAVIDSOHN: I wish to report a carcinoma of the thyroid which in long duration of disease resembled those reported by Dr. Miller. A woman aged 20, entered the Mount Sinai Hospital on March 24, 1937, because of a swelling of the neck which she had had for thirteen years. The parents refused operation. In 1926 an operation was performed at the Mayo Clinic, and the diagnosis of primary carcinoma of the thyroid was made. Thereafter the patient was given roentgen therapy once each year at the Mayo Clinic. In 1927 roentgenograms of the lungs demonstrated metastases in the lungs, and dyspnea developed. Myxedema appeared in 1930 and was treated with daily doses of desiccated thyroid. March 29, 1937, a tracheotomy was done because of dyspnea and profuse expectoration of purulent sputum, but gave only temporary relief. Death occurred March 31, 1937. The postmortem examination demonstrated a large amount of scar tissue, with only islets resembling thyroid gland. The cervical lymph nodes were large, and most of them were calcified. The trachea was compressed. The lungs and liver contained many metastases. Tissues removed at the Mayo Clinic were anaplastic adenocarcinoma. Those from the neck, lymph nodes, lungs and liver were differentiated adenocarcinoma.

O. T. SCHULTZ: The youth of some patients with carcinoma of the thyroid and the long time they live with the disease are remarkable. Marine reported that iodine is not stored in the carcinoma tissues.

B. H. NIEMAN: The enlarged thyroid of a patient at the Cook County Hospital was removed surgically and seemed benign. Later there were metastatic tumors in the skull and elsewhere.

JOSEPH L. MILLER JR.: Few, if any, cells in mitosis were observed.

MESENTERIC CYSTS IN CHILDREN. W. PRICE KILLINGSWORTH.

In a review of the literature on mesenteric cysts in children it was found that 210 cases in children under 15 years had been recorded, and to this number the present paper adds 44. The results of the detailed study of these 254 cases are given. Mesenteric cysts are more frequent than is usually believed. Approximately 750 cases are recorded in the literature, one third of which concerned children under 15 years of age. These cystic tumors in children have confusing clinical, surgical and pathologic features, none being pathognomonic. There is no agreement as to the etiologic background or as to the pathologic classification among various investigators. Mesenteric cysts occur equally in males and females. All races have them, although they are rare in the Negro. Sixty-two per cent of mesenteric cysts occur in children under 6 years of age, and half of this number, in children under 1 year of age. In 15.5 per cent of the cases reported in children no symptoms were noted. In the others the chief complaints were abdominal pain, vomiting and prostration (45 per cent), enlargement of the abdomen (26 per cent), variable abdominal pain alone (11.5 per cent) and symptoms not referable to the abdomen (2 per cent). The outstanding clinical or preoperative diagnoses in this group of cases were: unclassified abdominal tumor (30 per cent), intestinal obstruction (2.2 per cent), appendicitis (12 per cent), tuberculous peritonitis (7 per cent), intussusception (6 per cent), pyloric stenosis and volvulus (7 per cent each). No diagnosis was ventured in 8 per cent of the cases. As to location, 64 per cent were found in the mesentery of the ileocecal region, 10 per cent in the mesentery of the colon, 11 per cent in the mesentery of the ileum and jejunum and a few in the mesentery of the duodenum, omphalomesenteric region and under the serosa of the stomach. A palpable abdominal tumor was present in 65.5 per cent of cases. In 75 per cent of all cases the cysts were unilocular. Operative procedures were employed in 82 per cent of this series, with a mortality of 22 per cent, as compared with a total mortality of 40 per cent. Thirty-two per cent of these cysts were enteric, 20 per cent chylous, 13 per cent lymphatic and 8 per cent serous. Less than 8 per cent had fetal inclusions, omphalomesenteric or dermoid in nature. Less than 3 per cent were due to known bacterial or parasitic infections, and no pathologic diagnosis was made in 12 per cent.

Book Reviews

Enzyme Chemistry. By Henry Tauber, Ph.D. Cloth. Price, $3. Pp. 243. New York: John Wiley & Sons, Inc., 1937.

Tauber has succeeded in writing an admirably concise but comprehensive survey of the more recent and important researches in various fields of enzyme chemistry. To this subject contributions are being made with bewildering and unprecedented frequency, mostly of a supplementary nature but occasionally of a truly basic character. Brevity has been achieved not by mere compression but by the critical selection and balanced arrangement of material. Controversial subjects, such as biologic oxidation, concerning which the theories of Wieland and Warburg strive for supremacy, are treated judiciously, with the emphasis placed on the facts obtained by experimentation, in accordance with the tradition of Galileo, rather than on untenable theoretic assumptions. The author not only preserves for the reader the living spirit of a dynamic science but gives him a sense of proportion and skilfully guides him so that he will not lose himself in a mass of conflicting detail.

The broad scope of the volume is indicated by such chapter headings as the following: "Esterases," "Proteolytic Enzymes and Peptidases," "Oxidizing Enzymes," "The Flavin Oxidation System of Warburg and Christian and Its Relation to Other Dyes." At the end of each chapter is appended a useful and up-to-date bibliography of current literature.

Modern research in enzyme-chemistry has proceeded along two particular lines of inquiry, viz., the chemical nature of enzymes and their character and the cause of their peculiar mode of action, i. e., their specificity. Both of these problems appear to be intimately connected with each other and are adequately covered in the introductory chapter.

The methods of Northrop and his colleagues for the isolation and crystallization of pepsin and chymotrypsin and their precursors are presented, with the proof that crystalline pepsin, for example, is a protein on the ground that the only constituents so far demonstrated in it are amino acids.

It is gratifying to observe that the remarkable studies of Bergmann on the specificity of papain have been incorporated into the text. Unfortunately, there is no description of the excellent microchemical method of Linderstroem-Lang and Holter for the determination of certain enzymes in small amounts of tissue, a method which offers a new attack on problems of interest to the pathologist.

There is a brief discussion of the important role played by phosphatases in bone formation and of their significance in different bone diseases and in jaundice. Attention is drawn to the studies of Bodansky and Henry L. Jaffe, and it is pointed out that serum phosphatase is a more reliable index of the severity and the rate of healing of rickets than the serum calcium–inorganic phosphorus ratio.

For those interested in the role of enzymes in pathologic processes as well as for those desirous of bringing their knowledge of enzyme chemistry up to date, a reading of this excellent monograph, which contains much sound information within a small space, will be well repaid.

Report of the Medical Research Council for the Year 1936-1937. Committee of the Privy Council for Medical Research. Presented by the Lord President of the Council to Parliament by Command of His Majesty, February 1938. Paper. Pp. 195. Price, 3 shillings. London: His Majesty's Stationery Office, 1938.

The report summarizes well the activities of the Medical Research Council for 1936-1937. The first part deals with matters of special interest. In it is described the new scheme for extensive research in chemotherapy, which will be supported by a government grant of £30,000 annually. There is a review of the

results of the use of sulfanilamide in the treatment of streptococcic and gonococcic infections. There are sections on influenza, egg membrane culture of viruses, surgical operations on the heart, mental defects, the role of salt in the body, nicotinic acid as a vitamin, the diagnosis of cholera, the experimental study of leprosy and the organization and buildings of the National Institute for Medical Research. The work of the latter organization is reviewed in the second part, followed by a list of its publications during the year. Part 3 discusses the work on biologic standards. Next come reviews of the investigations of the clinical research units and of members of the external staff, of work aided by grants, of research in tropical medicine and of industrial health research. The last two sections consider traveling fellowships and the membership of the council. At the end are complete indexes of subjects, institutions and personal names. The foregoing enumeration shows the wide range of the work supported by the Medical Research Council, which by its allocation of government and other funds exerts a powerful influence on British medical research. One gets the impression of a large organization of investigators working in specialized groups. This is not the place to consider the results of the investigations, many of which are familiar through earlier publications. Any one who is interested in the progress of medical research will find this report interesting and instructve.

The American Illustrated Medical Dictionary: A Complete Dictionary of the Terms Used in Medicine, Surgery, Dentistry, Pharmacy, Chemistry, Nursing, Veterinary Science, Biology, Medical Biography, Etc., with the Pronunciation, Derivation and Definition. W. A. Newman Dorland, A.M., M.D., F.A.C.S., Lieut.-Colonel, M.R.C., U. S. Army. With the collaboration of E. C. L. Miller, M.D. Eighteenth edition. Fabrikoid. Pp. 1,607, with 942 illustrations. Price $7; thumb indexed $7.50. Philadelphia and London: W. B. Saunders Company, 1938.

This dictionary was published first in 1900. The successive editions have reflected well the growth of medicine and the related sciences. According to the preface, the present edition contains new as well as improved definitions on every page. In fact, more than 3,000 new words have been defined, and the volume has been enlarged by more than 60 pages. About a hundred new tests have been described. The plural forms of many words have been supplied, but some are still missing, e. g., for "actinomyces," "amygdala" and "sporothrix." The present edition contains 283 portraits. There are noteworthy omissions, e. g., Antony van Leeuwenhoek, Marie and Pierre Curie, Emile Roux, Emil A. von Behring, Clemens von Pirquet and on the American side William B. Beaumont, John S. Billings, Walter Brashear, Daniel Drake, Christian Fenger, Fielding H. Garrison, Oliver Wendell Holmes, William W. Keen, Joseph Leidy, Ephraim McDowell, William T. G. Morton, Walter Reed, Henry T. Ricketts, Benjamin Rush and Theobald Smith.

The Pathology of Diabetes Mellitus. Shields Warren, M.D. With a foreword by Elliott P. Joslin, M.D. Second edition, revised and enlarged. Cloth. Pp. 246, with 89 illustrations. Price $4.75. Philadelphia: Lea & Febiger, 1938.

Important advances have been made in knowledge of diabetes during the eight years since the first edition of this work appeared. Warren emphasizes new problems that now confront the pathologist by the following questions: "Are infants of diabetic mothers abnormally large, with hyperplastic islands of Langerhans? What is the nature of the hepatomegaly in diabetic children? What happens when a previously diabetic child becomes consistently hypoglycemic? What is the role of the pituitary? Why does arteriosclerosis in the diabetic person tend to pick out the coronary arteries and those of the legs? Has a given patient died of insulin overdosage or pulmonary embolus?" The book is based on extensive and thorough personal observations as well as on a study of the literature. It gives a succinct summary of knowledge of all the phases of diabetes that can be studied by morphologic methods. Pathologists and clinicians will find the book a most valuable guide in their work with diabetes.

Books Received

LES GROUPES SANGUINS. LEUR APPLICATION À LA BIOLOGIE A LA MÉDECINE ET AU DROIT. Professeur Ludwik Hirszfeld, directeur du département de bactériologie et médecine expérimentale de l'Institut d'Hygiene de l'état, à Varsovie. Translated from Polish by Mme. Hanna Hirszfeld. Paper. Pp. 169, with 15 illustrations. Price 15 francs. Paris: Masson & Cie, 1938.

ÉTUDES SUR LA RAGE. P. Remlinger, directeur de l'Institut Pasteur de Tanger, et J. Bailly, chef de service à l'Institut Pasteur de Tanger. Monographie de l'Institut Pasteur. Paper. Pp. 174. Price 40 francs. Paris: Masson & Cie, 1938.

LA MALADIE D'AUJESZKY. P. Remlinger, directeur de l'Institut Pasteur de Tanger, et J. Bailly, chef de service à l'Institut Pasteur de Tanger. Paper. Pp. 202, with 16 illustrations. Price 45 francs. Paris: Masson & Cie, 1938.

CLASSIC DESCRIPTIONS OF DISEASE. Ralph H. Major, M.D., Professor of Medicine, University of Kansas School of Medicine. Second edition. Cloth. Pp. 727, with 137 illustrations. Price $5.50. Springfield, Ill.: Charles C. Thomas, Publisher, 1938.

ANNUAL REPORT OF THE INSTITUTE FOR MEDICAL RESEARCH FOR THE YEAR 1937. A. Neave Kingsbury, Director, Institute for Medical Research, Federated Malay States, Kuala Lumpur. Pp. 174. Kuala Lumper: Federated Malay States Government Press, 1938.

ENDEMIC FILARIASIS IN THE FEDERATED MALAY STATES. J. Orde Poynton and E. P. Hodgkin. Bulletins from the Institute for Medical Research, Federated Malay States, no. 1 of 1938. Pp. 67. Kuala Lumpur: Federated Malay States Government Press, 1938.

PATHOLOGISCHE HISTOLOGIE. Dr. Max Borst, O. Ö. Professor der allgemeinen Pathologie und der pathologischen Anatomie an der Universität München. Third edition. Paper. Pp. 522, with 361 illustrations. Price 75 reichmarks. Berlin: Julius Springer, 1938.

ALICE IN VIRUSLAND. Paul F. Clark, Professor of Bacteriology, University of Wisconsin Medical School. Cloth. Pp. 23, illustrated. Price $1. Madison, Wis.: Society of American Bacteriologists, 1938.